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CONTENTS

PAGE

The Peripheral Blood Picture in Some Diseases of Animals. By A. R. Jennings 161

Investigations into Delayed Toxicity due to Dimidium Bromide. III. Some Experimental Observations in Ruminants. By M. L. Burdin and W. Plowright ... 178

Investigations into delayed Toxicity due to Dimidium Bromide. IV. Effect of High Dosage Levels of Dimidium Bromide in Cattle. By W. Plowright and M. L. Burdin 189

Studies on Experimental Streptococcal Mastitis. VII. Immunological Studies in Goats. By H. H. Holman, I. H. Pattison and W. S. Gordon 196

Chemotherapeutic Response of the Organism causing Enzootic Abortion in Ewes. By J. Francis 214

A Study of the *In Vitro* and *In Vivo* action of certain Antibiotics. By S. J. Edwards. 220

Studies on Vitamins A and C in Bovines. The Seasonal Variation in the Vitamin A Reserves of Cows. By J. Stewart, J. W. McCallum, and P. S. Watts ... 237

THE PERIPHERAL BLOOD PICTURE IN SOME DISEASES OF ANIMALS*

By

A. R. Jennings

Department of Veterinary Pathology, University of Liverpool.

INTRODUCTION

During the course of routine diagnostic work, a relatively large number of blood samples from animals affected with a variety of conditions was available for investigation. In order to obtain precise information on the course of a disease as revealed by changes in the blood picture, it is desirable to examine the blood at frequent intervals, but in veterinary practice, it is often difficult to obtain more than one sample of blood, and consequently many of the specimens received at the laboratory were single samples. Despite the obvious limitations of this material, haematological examinations were made in order to determine how far such samples were of value as an aid to diagnosis. It was felt that if relatively simple examinations of one or two specimens of blood proved to be significant, then such information would be of value to the busy practitioner. Some experimental work was also carried out in an effort to confirm the results obtained from the field samples.

METHODS

Techniques

Blood samples were taken into ringed tubes containing 3 mg. of dry potassium oxalate per ml. of blood. The examinations which were made as soon as possible after collection comprised total red and white cell counts, haemoglobin determinations, packed cell volumes, sedimentation rates (not all cases), differential leucocyte counts, Arneth counts, platelet counts and, in a few cases, reticulocyte counts. The techniques used were those described by Wintrobe (1946).

For comparative purposes the normal standards were those given by Holman (1944) for the horse, ox and dog, while the standards suggested by Jennings (1947) were used for the cat.

Experimental Observations

The following table indicates the number of samples investigated, together with the number of cases of each disease.

* Part of a thesis for the degree of M.V.Sc., University of Liverpool.

TABLE I

Species	*Disease*	*Cases*	*Samples*
Horse	Influenza	5	12
	Strangles	6	19
	Purpura Haemorrhagica ...	16	38
	Poll Evil	4	16
Ox	Traumatic Reticulitis	5	7
	Parasitic Gastro-enteritis ...	8	8
	Pyelonephritis	3	4
	Congenital Anasarca	2	4
Dog	Non-parasitic skin Disease ...	14	18
	Leptospirosis	10	11
Cat	Feline Enteritis	30	52
	Feline Influenza...	28	85

DISEASES OF THE HORSE

Equine Purpura Haemorrhagica

Sixteen cases of purpura were investigated and a total of 38 samples examined. Many were associated with equine influenza. The means and the standard deviations of the means were as follows: —

Red cell count	$5.94 \pm 0.27 \times 10^6$ per c.mm.
Haemoglobin	9.89 ± 0.83 g. per cent.
Packed cell volume	26.47 ± 3.52 per cent.
M.C.V.	47.7 cubic micra.
M.C.H.C.	38.2 per cent.
Platelet count	$261.1 \pm 13.76 \times 10^3$ per c.mm.
White cell count	$13.76 \pm 2.17 \times 10^3$ per c.mm.
Neutrophils	75.36 ± 12.54 per cent.
Lymphocytes	15.72 ± 8.25 per cent.
Monocytes	7.13 ± 3.53 per cent.
Eosinophils	1.60 ± 1.60 per cent.
Basophils	0.13 ± 0.13 per cent.

It is proposed to deal in detail with Case No. 1 since the clinical condition of this animal was carefully noted and also a series of examinations was carried out.

At the commencement of the disease the animal had a temperature of 104°F. and severe respiratory distress. The hind limbs were swollen; there was a haemorrhagic nasal discharge and the nasal mucosa showed numerous petechiae. During the next three days oedematous areas appeared between the forelegs and around the muzzle. During this period the haemoglobin level and the packed cell volume showed a constant drop. The total white cell count fell from an initial figure of 16,000 to 12,800 cells per c.mm. but the

absolute neutrophil count showed a steady rise. As might be expected, there was an eosinopenia during this neutrophilic response. The eosinophils reappeared in the peripheral blood on the fifth day at the height of the neutrophilic response and immediately before the latter began to wane. Clinically the animal showed marked improvement; the appetite had returned and the animal was much "brighter". On the ninth day after the commencement of the attack the horse was clinically normal.

All the fatal cases in this series had initial low red cell counts and haemoglobin values; moreover, with the exception of No. 16 they never developed leucocytosis. In the relatively mild cases, however, there was a marked leucocytosis. In this connection it is considered that counts of 14,000 and over, i.e. three times the standard deviation of the normal mean, indicate a significant rise in circulating leucocytes. In one case (No. 3), eosinophils had not reappeared in the circulating blood and there was no monocytosis, although clinically the animal was almost normal, the acute stage having quickly subsided. Thus, with the exception of the increase in lymphocytes, there was no evidence of the typical monocytic defence phase and the eosinophil convalescent phase. Throughout this series of cases the monocytes did not appear at any constant stage; in some cases they were present throughout the disease, e.g. No. 2, and in others, e.g. No. 13, they were absent or very sparse during blood examinations.

The overall impression revealed by these examinations was that in equine purpura haemorrhagica there was a normocytic, normochromic anaemia with a tendency towards a macrocytic type since the M.C.V. (47.7 cubic micra) was at the upper end of the normal range. The mean platelet count indicated that the purpura was of the non-thrombocytopenic type. In the non-fatal cases there was a well-marked leucocytosis due to neutrophilia.

Witman and Contis (1924) described the blood changes as those of a progressive anaemia. They said that the leucocytes increased in number with a relative lymphopenia, and claimed that the platelets disappeared. Hutyra, Marek and Manninger (1938) stated that the blood picture was not characteristic. They considered that haemolysis, thrombocytopenia and an increased sedimentation rate were features of the disease. Bellocq and Louf (1943) described an increased fragility of the red cells and a lowered platelet count. Jennings and Highet (1947) concluded that purpura in the horse was of the non-thrombocytopenic type. There was an anaemia which was related to the severity of the disease, a well-marked neutrophilia and a shift to the left. Biggers, Ingram and Murray (1949) agreed that the condition was a non-thrombocytopenic purpura. They stated that the blood clotting mechanism was normal and that there was anaemia. Rapid rises in the red cell counts were regarded as due to periods of haemoconcentration following loss of plasma constituents into the tissues. The leucocytic changes were

discussed and found to be similar to those noted by previous workers.

The results of this investigation are in general agreement with those of previous workers. The initial low red cell counts have not previously been stressed, although Biggers, *et al* (1949) encountered one fatal case out of three with a low initial red cell count. The cause of the oligocythaemia is unknown. It was felt that the haemorrhages were insufficient to reduce the red count and that the lowering of the red cells was probably related to previous disease. The work of Biggers *et al* supports this theory. On the other hand, these workers stressed the presence of a polycythaemia which they said was due to loss of plasma volume, whereas in this series, no such haemoconcentrations have been noted.

Poll Evil

Four cases of poll evil were investigated and sixteen samples examined. In three, the lesions were "open" and had been in existence for some time prior to the first blood examination. No. 1 was a closed case which appeared to resolve, but unfortunately examinations were made after the horse had been treated with a *Br. abortus* (dead) vaccine, and this factor must be borne in mind when assessing the blood picture. Surgical interference had taken place in Nos. 2 and 4 before blood examinations were made.

The means and the standard deviations of the means were as follows: —

Red cell count	$7.55 \pm 1.79 \times 10^6$ per c.mm.
Haemoglobin	9.41 ± 0.72 g. per cent.
White cell count	$17.1 \pm 3.3 \times 10^3$ per c.mm.
Neutrophils	71.56 ± 3.26 per cent.
"Non-segmenters"	30.86 ± 13.0 per cent.
Lymphocytes	22.12 ± 9.27 per cent.
Eosinophils	2.31 ± 2.31 per cent.
Monocytes	2.0 ± 1.77 per cent.

Owing to unavoidable circumstances the packed cell volume was determined in one case only, and therefore is not included in the table of mean values given above.

At the time of the first blood examination of case No. 2 the horse had been affected with poll evil for some weeks and had been operated upon ten days before the blood sample was taken. Other blood samples were taken during the next 17 days. At the first examination there was a leucocytosis (14,500 cells per c.mm.) but the red cell count and the haemoglobin content were normal and surprisingly high in view of the extensive blood loss during surgical interference. Up to the fifth day the red cells and the haemoglobin values declined steadily but were never outside the normal range.

During this time the absolute neutrophil count dropped, there was a rise in the circulating eosinophils but the lymphocytes decreased as did the monocytes. Commencing on the fifth and extending to the ninth days there was a marked neutrophilic "invasion" phase with a simultaneous eosinopenia. The total white cell count increased over this period and reached a maximum of 21,000 cells per c.mm. on the ninth day. Clinical records of the animal showed a slight rise in temperature on the seventh and ninth days respectively. On the eleventh day the wound was opened and several small abscess areas and sinuses were treated. A fairly clear defence stage was seen as judged by the drop in the neutrophils and the increase of the eosinophils.

Case No. 1 was a "closed" case which had received vaccine treatment before blood samples were taken. This horse had a leucocytosis for the first five days with an increasing neutrophilia and a shift to the left. The leucocytic response never exceeded that of the three open cases and on the eighteenth day the peripheral blood picture was normal. It was probable that in this case the leucocytosis was due mainly to the use of the heat killed suspension of *Br. abortus*.

Horse No. 4 was the most severe case and had undergone two very extensive surgical operations. This animal had a post-haemorrhagic anaemia. The mean corpuscular haemoglobin concentration was 44.5 per cent. and the mean corpuscular volume 59.6 cubic micra; these figures indicated a macrocytic anaemia. The leucocytic response was very poor as compared with case No.2.

No references to the blood picture in poll evil have been found, although Connor (1945) described the changes in equine pyogenic infections including fistulous withers and quittor cases. The findings here resemble closely those described by him in fistulous withers.

Equine Influenza

Five cases of a mild form of influenza were investigated. The more severe cases in which there was a tendency for purpura haemorrhagica to develop have been dealt with under that heading.

The mean findings and the standard deviations were as follows:

Red cell count	$6.66 \pm 0.66 \times 10^6$ per c.mm.
Haemoglobin	10.3 $\pm$0.6 g. per cent.
Sedimentation rate	23.2 $\pm$4.0 mm. per hour
White cell count	$11.35 \pm 3.36 \times 10^3$ per cent.
Neutrophils	68.20$\pm$9.16 per cent.
Lymphocytes	26.60$\pm$5.38 per cent.
Monocytes	3.50$\pm$1.49 per cent.
Eosinophils	3.08$\pm$3.08 per cent.

It will be seen that there was no change in the blood picture which was of value in diagnosis, but in case No. 1 there was a significant leucocytosis and neutrophilia in the early stages of the disease, together with the usual eosinopenia and lymphopenia.

Maurer and Jones (1943) investigated the blood picture in two main groups of cases. In the uncomplicated virus disease there was leucocytosis due to a relative and absolute neutrophilia. In those cases in which there was a secondary bacterial multiplication there was a diphasic temperature reaction and a severe neutrophilia, which reached its peak during the second febrile stage. During recovery there was a monocytosis and a lymphocytosis. Witte (1942) described the anaemia present in equine influenza as microcytic in type. Neuman-Kleinpaul and Myers (1936) stated that there was an accelerated sedimentation rate, and this observation was confirmed by Cordier and Menager (1938).

Strangles

Six cases of strangles were investigated and 19 samples were examined.

The mean value of the readings and their standard deviations were as follows:

Red cell count	$7.22 \pm 2.11 \times 10^6$ per c.mm.
Haemoglobin	10.33 ± 0.50 g. per cent.
Sedimentation rate	26.10 ± 6.70 mm. per hour.
White cell count	$14.13 \pm 4.14 \times 10^3$ per c.mm.
Neutrophils	67.21 ± 6.10 per cent.
"Non-segmenters"	28.78 ± 7.45 per cent.
Lymphocytes	26.21 ± 4.49 per cent.
Monocytes	3.68 ± 0.56 per cent.
Eosinophils	2.60 ± 2.03 per cent.

It was possible to follow the blood picture throughout the disease in one case only (No. 5). The blood changes were those which might have been expected namely a peak neutrophilia and a strong shift to the left at the time of the pointing of a submaxillary abscess which was opened surgically on the fifth day, resulting in a reversion of the blood leucocyte count to the normal by the ninth day.

Burnett (1917) stated that in mild cases the red cell count was not changed but that in the acute febrile disease there was either a relative polycythaemia or "a moderate anaemia" with "a greater relative diminution of haemoglobin." The anaemia in equine strangles has been ascribed either to streptococcal haemolysins or to a dyshaemopoiesis or to both factors (Burnett 1917; Krupski, 1931; and Eberbeck and Hemmert-Halswick, 1944). These workers, together with Hutyra *et al* (1938) and Connor (1945) also described leucocytosis and neutrophilia. Mielke and Wirth (cited by Hutyra

et al, 1938) described a leucocytosis with a relative lymphopenia and eosinopenia in acute strangles. The anaemia was usually normocytic in type and due largely to streptococcal haemolysins; there were suggestions that it might be dyshaemopoietic.

In this series of cases there was no evidence of any anaemia, the figures for the red cells and the haemoglobin levels lying within the normal range. The changes in the leucocyte counts have been confirmed since all the cases, at some stages of the disease, developed white cell counts of 14,000 per c.mm.

DISEASES OF CATTLE

Traumatic Reticulitis and Pericarditis

Five cases of traumatic reticulitis were investigated and a total of seven samples examined. The diagnosis was confirmed either at post-mortem examination or by rumenotomy.

The mean values and their standard deviations were as follows: —

Red cell count	$6.61 \pm 1.81 \times 10^6$ per c.mm.
Haemoglobin	10.01 ± 0.4 g. per cent.
P.C.V.	32.25 ± 6.5 per cent.
White cell count	$15.3 \pm 3.62 \times 10^3$ per c.mm.
Neutrophils	68.57 ± 9.27 per cent.
"Non-segmenters"	20.28 ± 4.82 per cent.
Lymphocytes	24.7 ± 5.71 per cent.
Monocytes	3.14 ± 2.08 per cent.
Eosinophils	3.14 ± 1.64 per cent.

These figures show significant changes in the white cells. There was a leucocytosis (over 14,000 cells per c.mm.) with a neutrophilia and a marked shift to the left together with an eosinopenia and a lymphopenia.

Arthur (1946) confirmed earlier work on the blood pictures in these conditions. In traumatic reticulitis there was said to be a marked leucocytosis with neutrophilia. The neutrophils varied from 30 per cent. to 57 per cent. of the total leucocytes and of these the unsegmented neutrophils varied from 8 per cent. to 32 per cent. There was also a marked eosinopenia. Cases of traumatic pericarditis showed an even greater degree of neutrophilia since the neutrophils accounted for as much as 78 per cent. of the total leucocytes. The shift to the left was also more marked and smears showed "band" forms ranging from 22 to 66 per cent. of the total neutrophils. Arthur regarded these blood changes together with the clinical symptoms as diagnostic.

These results confirm the work done by Arthur (1946) and by earlier investigators. The main value of the findings lies in their application in the differential diagnosis of some forms of bovine "indigestion."

Congenital Anasarca

The blood picture of this peculiar condition seen in Ayrshire calves has not previously been recorded. Two female calves belonging to a pedigree attested Ayrshire herd were available for study. The dams were not related but the calves, which were sired by the same bull, were well developed at birth but both had subcutaneous oedema of the fore limbs and intermaxillary spaces. The oedema increased during the first week of life and when aged eight days they both had very extensive anasarca. Each animal also had an umbilical hernia.

The mean haematological data in two cases (four samples) were as follows: —

Red cell count	5.98×10^6 per c.mm.
Haemoglobin	10.37 g. per cent.
White cell count	5.08×10^3 per c.mm.
Neutrophils	25.75 per cent.
Lymphocytes	65.0 per cent.
Monocytes	7 per cent.
Eosinophils	2.25 per cent.

These figures all fall within the normal pattern given for healthy calves, although the mean white cell count is at the lower end of the range.

Detailed biochemical, morbid anatomical and histopathological examinations were carried out. The only detectable abnormality was, in each case, a low plasma protein content.

Bovine Parasitic Gastro-enteritis and Parasitic Pneumonia

The eight cases of bovine parasitism investigated were all severe and all came to post-mortem examination. In each case, *Dictyocaulus viviparus* and *Haemonchus contortus* were present. The mean strongyle egg count was 1,250 per g. of rectal faeces. The haematological examinations were made immediately before the animals were slaughtered.

The detailed findings may be seen in Table 2.

The mean figures and their standard deviations were as follows: —

Red cell count	$4.25 \pm 0.30 \times 10^6$ per c.mm.
Haemoglobin	8.48 ± 1.5 g. per cent.
P.C.V.	24.12 ± 1.74 per cent.
White cell count	$9.07 \pm 2.77 \times 10^3$ per c.mm.
Neutrophils	45.5 ± 5.95 per cent.
"Non-segmenters"	14.3 ± 6.71 per cent.
Lymphocytes	48.12 ± 6.71 per cent.
Monocytes	1.85 ± 1.58 per cent.
Eosinophils	4.12 ± 3.03 per cent.

TABLE II

BLOOD CELL COUNTS IN BOVINE PARASITIC GASTRO-ENTERITIS AND PARASITIC PNEUMONIA

No.	*RBC*	*WBC*	*Hb.*	*N*	*L*	*M*	*E*	*B*	*NS.*	*PCV*
1	3·6	6·0	8·2	55	35	5	5	0	20	25
2	4·6	8·4	11·4	42	54	4	0	0	20	20
3	5·2	7·0	10·4	40	58	2	0	0	—	30
4	4·5	11·5	7·9	45	50	0	5	0	15	30
5	5·2	15·2	7·4	50	45	0	5	0	12	25
6	3·9	8·5	7·5	45	45	1	9	0	10	20
7	2·9	7·1	7·1	48	45	0	2	0	—	20
8	4·5	8·9	8·0	40	53	3	7	0	9	25

The mean values for the haemoglobin, red cell counts and packed cell volume were below the normal mean but were not pathognomic. Examinations of individual animals showed No. 7 to be the most badly affected. This cow had a M.C.V. of 68.8 cubic micra and M.C.H.C. 32 per cent. The former figure was well outside the normal range and indicated a macrocytosis. Nos. 1, 4, 5 and 6 had low red cell counts, haemoglobin levels and lowered values for the packed cell volumes. No. 1 had a M.C.V. of 66.6 cubic micra (indicating large cells) and the M.C.H.C. was 32.8 per cent. Cases 4 and 5 had values respectively of M.C.V. 66.6 cubic micra, M.C.H.C. 29.3 per cent, and M.C.V. 44.2 cubic micra and M.C.H.C. 29.6 per cent.

The mean value for the total white cell count was within the normal range. One animal, No. 5, developed a leucocytosis (15,200 cells per c.mm.) with a neutrophilia. All the animals showed a bias towards a neutrophilia, the percentage of neutrophils being at the upper end of the normal range. There was no evidence of an eosinophilia, in fact the reverse was noted. In all the cases the eosinophil counts were towards the bottom of the normal range.

Work done on the blood picture of parasitic diseases in ruminants has been largely confined to sheep. The picture in cattle presented above is not unlike that seen in sheep and described by Holman and Pattison (1941) who suggested that the macrocytosis might be due to dyshaemopoiesis. Fourie (1931) also described a macrocytic anaemia in haemonchosis in sheep but he attributed this to a post-haemorrhagic anaemia. Delaune (1939) noted anaemia and "constant leucocytosis" in parasitised cattle.

It appears, therefore, that in parasitic gastro-enteritis and parasitic pneumonia in cattle there may be a macrocytic anaemia and a variable leucocytosis with a neutrophilia, and an eosinopenia.

DISEASES OF THE DOG

Non-Parasitic Skin Disease

Fourteen cases of dry eczema were investigated; clinically they all appeared to be similar with lesions on the face and abdomen. Microscopic examinations showed no evidence of skin or intestinal parasites. The samples were taken before any treatment was started.

The detailed findings may be seen in Table 3.

The mean results and their standard deviations were as follows: —

Red cell count	$5.61 \pm 2.04 \times 10^6$ per c.mm.
Haemoglobin	10.91 ± 1.07 g. per cent.
White cell count	$10.01 \pm 3.04 \times 10^3$ per c.mm.
Neutrophils	62.61 ± 10.53 per cent.
Lymphocytes	24.88 ± 7.89 per cent.
Monocytes	3.44 ± 2.24 per cent.
Eosinophils	8.61 ± 5.50 per cent.

Table III

Blood Cell Counts in cases of Canine Skin Disease

No.	*RBC*	*WBC*	*Hb.*	*N.*	*L.*	*M.*	*E.*
1 ...	5·22	6·8	11·0	65	25	4	6
	5·36	7·9	11·3	64	17	5	4
2 ...	5·0	5·0	10·5	44	40	6	10
	6·5	8·5	13·6	45	30	5	20
	6·1	9·7	12·6	51	36	3	10
3 ...	5·6	10·5	10·0	62	28	1	9
4 ...	6·3	12·0	11·7	60	35	2	3
5 ...	3·51	20·0	8·0	60	20	7	13
6 ...	5·0	10·0	11·0	60	30	2	8
7 ...	5·85	7·5	11·8	54	24	3	19
8 ...	6·0	12·0	10·7	70	13	2	14
9 ...	7·3	9·6	12·0	66	15	8	8
10 ...	6·0	7·0	12·0	80	15	1	4
11 ...	4·1	8·0	10·2	85	10	2	2
12 ...	6·6	10·0	10·5	70	27	1	2
13 ...	7·0	15·0	10·5	68	25	4	3
14 ...	3·6	12·8	7·5	70	25	0	5

The mean values for the red cells and the haemoglobin levels were within the normal ranges. Case No. 14, however, did show anaemia with a haemoglobin value of 7.5 g. per cent., i.e. a figure well outside the absolute minimum normal.

The mean white cell count was within the limits of normality although one of the cases showed evidence of leucocytosis. The mean neutrophil figure was at the lower end of the normal scale. One case, No. 11, had a bias towards a neutrophilia, but in the remainder it was in the other direction. Case No. 2 showed a well-marked neutropenia with neutrophil counts below 53 per cent. The figures for lymphocytes and monocytes were within the normal ranges.

In general, the eosinophils showed significant changes since the mean eosinophil percentage was 8.61 ± 5.5, which was well outside the normal range. In five cases the figure exceeded the maximum normal range and in seven, the count was in the "probable" pathological range. As might be expected, the eosinophils increased at the expense of the neutrophils, and this was well shown in Case No. 2.

No references to the blood picture of non-parasitic skin disease in the dog have been found. Holman (1944) stated that an eosinophilia was often noted in chronic skin diseases and diseases of the mucous membranes. Eosinophilia is often a feature of some forms of allergy and it may well be that some of these conditions were allergic in origin. The blood picture in these cases differed from that seen in parasitic disease in that there was an eosinophilia and a neutropenia.

Leptospirosis

Ten cases of leptospirosis were investigated and a total of eleven samples examined. The cases were confirmed as leptospiral infections by serological, histological, and biological examinations.

The mean values and their standard deviations were as follows: —

Red cell count	$5.68 \pm 0.31 \times 10^6$ per c.mm.
Haemoglobin	8.80 ± 2.12 g. per cent.
White cell count	$16.79 \pm 3.51 \times 10^3$ per c.mm.
Neutrophils	67.27 ± 6.79 per cent.
"Non-segmenters"	12.33 ± 4.08 per cent.
Lymphocytes	27.36 ± 5.71 per cent.
Monocytes	3.09 ± 2.54 per cent.
Eosinophils	1.63 ± 2.30 per cent.

Eight of these ten cases died before further blood examinations could be made and in the remaining two the owners failed to bring the animals back for examination. It was therefore impossible to correlate accurately the peripheral blood picture with the stage of the disease and only the general trends of the cellular reaction can be noted.

The mean values of the series failed to reveal any evidence of anaemia but in two cases (Nos. 4 and 5) the haemoglobin values of 7.3 and 7.5 g. per cent. respectively might be regarded as indicating a hypochromic anaemia. The mean white cell count was at the upper end of the normal range. Some cases (Nos. 5, 6 and 10) had white cell counts indicating a leucocytosis but one case (No. 1) showed a leucopenia. This dog had also a low red cell count at the same time and was seriously ill. Other cases showed an increase in the numbers of circulating neutrophils with a relative lymphopenia, but in none of them could the neutrophilic increase be regarded as exceeding the wide limits of normality.

Buchanan (1927) described a severe anaemia in leptospirosis whereas Monlux (1948) "failed to find any significant changes in the red cells except for an increased sedimentation rate." Eight of these 10 cases reported above support the findings of the latter worker. Monlux also stated that a leucocytosis due to a neutrophilia began on the third day after infection, reached a peak on the eighth day and then subsided. The results presented here do not, however, agree entirely with those of Monlux since there was an absolute lymphopenia in only one case (No. 1). They are also at variance with the findings of Buchanan who described a leucocytosis due largely to a lymphocytosis since, in the present series, a lymphocytosis was noted in one case only (No. 10).

DISEASES OF THE CAT

Feline Enteritis

Thirty cases of feline enteritis were investigated and blood samples were examined at varying stages during the natural and experimental diseases.

The mean red cell count, as estimated from 46 samples, was 6.08 millions per c.mm. with a standard deviation of 0.3. This mean figure is below that given for healthy kittens and the difference is statistically significant ("t" test). In some individual cases the red cell count was above normal and increased during the course of the disease. This relative polycythaemia was thought to be consequent upon dehydration due to the vomiting and diarrhoea. The general reduction in the number of red cells, although significant, was not perhaps as great as one might expect, considering the aplastic type of marrow which is seen in this condition. This apparent anomaly may be explained by the long life of the individual erythrocyte. The animal would either be dead or recovered before the effects of dyshaemopoiesis were apparent.

The mean haemoglobin level of 46 samples was 10.04 ± 0.38 g. per cent. The difference between these figures and the normal is significant. As it was not possible to determine the packed cell volume in all cases, the M.C.V. and the M.C.H.C. could not be determined, but the M.C.H. was calculated and found to be 16.34 micromicrograms.

The leucocytes showed very marked and significant changes. A total of 52 samples was examined and the mean white cell count which was 6.93 ± 3.53 thousands per c.mm. indicated a leucopenia. The decline in the numbers of leucocytes was rapid. In some cases the leucopenia began before the animal showed clinical evidence of the disease. This observation might have some value in the detection of early cases, but care would have to be taken in the interpretation of the findings, because of the variations in healthy kittens.

The differential leucocyte counts in 51 samples were of great value in showing the types of cells involved. The mean values were as follows:

Neutrophils	47.07 ± 8.1 per cent.
Lymphocytes	42.03 ± 7.49 per cent.
Monocytes	7.13 ± 4.51 per cent.
Eosinophils	3.60 ± 2.50 per cent.

An analysis of these results shows a neutropenia, a lymphocytosis and a monocytosis. The differences between these counts and those from normal animals were significant. The extent and severity of the neutropenia were parallel with the severity of the condition.

Hammon and Enders (1939) described a leucopenia and

ascribed it to a failure of leucopoiesis. They said that there was an early lymphopenia, followed by a panleucopenia due, they thought, to the action of the virus on the haemopoietic tissues. Recovery was marked by a leucaemoid response. Syverton, Lawrence, Ackart, Adams, Ervin, Haskins, Saunders, Stringfellow and Wetrich (1943), considered the disease to be an agranulocytosis, since there was a marked leucopenia with a relative lymphocytosis. Riser (1947) described a progressive anaemia and a leucopenia in experimental cases of the disease. He considered that all the blood cells decreased in numbers as the crisis was reached, at which point the animal either died or made a rapid recovery.

The findings in this series of cases resemble most closely those of Syverton *et al* (1943). The disease was essentially an agranulocytosis, with a relative lymphocytosis and monocytosis. There was a slight anaemia, and, in some cases, a relative polycythaemia.

Feline Influenza

In the present study the bloods of 28 affected cats were examined. The diagnosis of feline influenza was made on clinical, histopathological and haematological grounds, according to the standards suggested by Jennings (1949).

The mean red cell count of a total of 64 samples was 6.19 ± 2.11 millions per c.mm. The mean haemoglobin level for 61 samples was 10.82 ± 1.5 g. per cent. These figures are within the normal ranges and there was no evidence of anaemia.

The mean total white cell count obtained from a series of 61 samples was 18.85 ± 3.66 thousands per c.mm. It was above the normal and the difference was significant. Individual cases exhibited marked variations in the numbers of white cells; cases with bilateral conjunctivitis showed a very significant leucocytosis with counts exceeding 20,000 cells per c.mm. In mild cases with little involvement of the eyes, there was only a small leucocytic reaction.

The mean figures for the differential leucocyte counts were as follows: —

Neutrophils	63.62 ± 6.67 per cent.
Lymphocytes	25.36 ± 7.52 per cent.
Monocytes	7.20 ± 2.36 per cent.
Eosinophils	3.62 ± 2.40 per cent.

These figures show a highly significant neutrophilia and lymphopenia. There was also a significant monocytosis.

As the animals passed from the acute stages to the stages of recovery, the eosinophils and monocytes increased in numbers although the lymphocytic recovery was slow. The best guide in prognosis was the behaviour of the neutrophils as fluctuations in the clinical condition were ushered in by neutrophilic changes. In severe and fatal cases, in which death was due to broncho-pneu-

monia, the neutrophils showed toxic granulations and large numbers of "smudge" cells were observed.

The presence of a monocytosis was confirmed together with significant leucocytosis due to a neutrophilia in the acute stages of the disease. The red cells showed no significant changes. Hindle and Findlay (1932) had noted a slight and inconstant monocytosis in a disease they called feline distemper.

DISCUSSION

Berg (1945) showed that in a red cell count the distribution error may be ±10 per cent., and it may be even greater in the leucocyte counts. If, therefore, the mean count of the normal animal is used as a standard, wrong interpretations are almost inevitable. In an extreme case the healthy animal examined twice in one day may apparently reveal first a polycythaemia and then anaemia. Again, the animal may have "leucocytosis" one day and "leucopenia" the next. It is therefore necessary to establish not only the mean but the standard deviation of the mean unless errors are taken as inevitable.

In the analysis of the changes in the cellular elements the "t" test for the difference in means was used for the feline diseases since the numbers of animals used to determine the normal means were known. In the other cases twice the standard deviation each side of the normal mean was used and served as a useful guide to indicate a probable pathological range. Three times the S.D. indicated a definite pathological figure. This is a useful method where small numbers of samples have to be examined.

In the survey of the literature there were many gaps and many apparent discrepancies. Several workers failed to take in account the wide range of the "normal" animal. For example, Kernkamp (1939) describing "leucopenia" in swine fever stated that "white cell counts below 8,000 cells per c.mm. were diagnostic." The normal mean white cell count in the pig is 14.7 with a standard deviation of 4.5 thousand cells per c.mm. This indicates a very wide normal range and a count of less than 5.7 would be required before it could be regarded as significant. A failure to take into account physiological variations may also be responsible for wrong interpretations.

In the literature and in textbooks one finds constant reference to parasites causing a circulatory eosinophilia. Examination of the data does not substantiate this statement. The present survey indicates that there is usually a bias towards neutrophilia and a consequent circulatory eosinopenia. In mild cases there may be no change in the peripheral blood picture or there may be periods of circulatory eosinophilia followed by eosinopenia as the eosinophils are withdrawn into the parasitised tissues.

In some of the diseases of the horse fairly well marked stages in the course of the disease were reflected in the peripheral blood

picture. This might have been of value in prognosis in some instances, but this was not so in all cases, e.g. in purpura haemorrhagica the monocytes increased in all stages of the disease and not merely in the "defence" stage. Again in some mild cases of the disease there was an eosinopenia throughout the examinations. In one case of strangles the eosinophils increased during the "invasion" stage when one would have expected an eosinopenia. Anaemia was not a prominent feature of the equine diseases investigated, but there was a tendency for the haemoglobin levels to fall during the course of the disease. This is of interest since it is in accordance with the findings of Robscheit-Robbins and Whipple (1936), who noted that haemoglobin formation was inhibited in infectious or inflammatory processes.

In the canine diseases investigated the eosinophilia noted in the non-parasitic skin disease group had not previously been reported. The neutropenia present in these cases was perhaps unusual since many of these dogs had slight sepsis of the skin. The presence of an eosinophilia supports the view that many cases of canine skin disease may be allergic in origin.

The cases of canine leptospirosis demonstrated how much the peripheral blood picture could vary in different animals. The changes noted were not diagnostic and might be confused with many other conditions.

The blood pictures in the two feline diseases investigated were quite distinct. In feline enteritis there was an agranulocytosis whereas in feline influenza there was a leucocytosis due to a neutrophilia.

The general result of this work indicated that examinations of single samples was of very little value in view of (a) the very wide normal range of the blood cells, (b) the changes which occur during the course of any one disease, and (c) the possible experimental error. It became obvious that a series of examinations of diseased animals together with representative samples from the herd or flock would be necessary before clear-cut opinions could be expressed. In cases of single samples the very greatest care should be exercised before drawing any conclusion.

The main value of blood examinations would appear to be as an aid to prognosis but here again there are many drawbacks. The blood changes during the different phases of infection are by no means sharply defined and bearing in mind the experimental error, the peripheral blood picture may not accurately indicate the body's reaction.

SUMMARY

Examinations of single or multiple blood samples have been made in a variety of diseases of domestic animals.

The total peripheral blood picture is of no diagnostic significance except in the case of the anaemias.

The leucocyte picture may, in conjunction with the clinical picture, be of some value as an aid to prognosis.

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For reasons of space, it has not been possible to publish full details of each case. These figures will be made available on request to the Department of Veterinary Pathology, University of Liverpool.

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INVESTIGATIONS INTO DELAYED TOXICITY DUE TO DIMIDIUM BROMIDE

III. Some Experimental Observations in Ruminants

By

M. L. Burdin and W. Plowright

Veterinary Research Officers, Veterinary Laboratory, Kabete, Kenya.

INTRODUCTION

The occurrence in Kenya of toxic symptoms in cattle following mass-inoculation of dimidium bromide as a prophylactic against trypanosomiasis, has been described in a previous communication (Thorold and Plowright, 1952). This has led to an investigation of the toxicity of dimidium bromide at the Kenya Veterinary Laboratories, Kabete (Plowright, Burdin and Thorold, 1952).

Previous workers (Randall and Beveridge, 1946, 1947; Randall and Laws, 1947; Bell, 1945, 1947; Wilson, 1948; Wilde, 1949) have noted liver damage at post-mortem examinations after using the drug at various dosage levels which produced delayed toxicity with typical skin lesions. The work recorded in this paper has been mainly concerned with the determination of liver dysfunction during life in treated animals which have not shown skin lesions.

TECHNIQUE

General and Clinical

In the field, native stock seem to have been most frequently and most severely affected. Native Boran cattle were used exclusively to make the findings of wider application in East Africa.

The 24 bovines used were two-year-old, non-pregnant heifers and they were kept out at pasture continuously without supplementary feeding. They were dipped once a week in a Gammexane wash, weighed and divided into four groups of six individuals. After a preliminary period of observation, including two bleedings, three of the groups were injected on 12th December 1950 with dimidium bromide, batch 80, in 2 per cent. solution.

Group I received 1 mg./Kg.; 3 animals subcutaneously and 3 intravenously. Group II received 2 mg./Kg.; 3 animals subcutaneously and 3 intravenously. Group III received 3 mg./Kg.; 3 animals subcutaneously and 3 intravenously. Group IV comprised 6 untreated controls. Thereafter all animals grazed together and were under constant observation by a herdboy. They were bled and weighed weekly, and were clinically examined in the crush twice a week.

The dimidium bromide was made up with boiling water and injected within an hour of preparation. In latter experiments different batches of the drug have usually been pooled to eliminate

any possible variation between samples. In this connection six different batches were obtained and no difference was found between them in melting point or solubility, except for one batch with a 0·2 per cent. water-insoluble residue. Differences in appearance and colour were probably due to varying fineness of grinding. Makers now recommend only the subcutaneous and intravenous routes of injection which have been used throughout these studies.

Biochemical

The following blood examinations have been performed:— (i) Haemoglobin by the method of Newcomer (1923). (ii) Serum Alkaline Phosphatase by the method of Gutman and Gutman (1940), the results being expressed as King and Armstrong units (King and Armstrong, 1934). (iii) Non-protein nitrogen by King's method on whole blood. (iv) Van den Bergh reaction on serum by the method described by Kolmer and Boerner (1945). (v) Fouchet reaction as described by Beaumont and Dodds (1947).

Weekly bleedings were taken into chemically clean McCartney bottles for serum and in tubes with 2·0 mg./ml. dry sodium oxalate for whole uncoagulated blood. Samples were stored in the refrigerator and estimations carried out, whenever possible, within two days of collection.

The Hanger's cephalin-cholesterol flocculation test was tried but gave a positive reaction within one hour in 24 normal bovine and 10 normal goat sera. The probable reason was that the high proportion of globulin in bovine sera had a precipitating effect in excess of the protective properties of the albumin. Other similar reactions, e.g., Takata and Ara and Colloidal gold have not been tried but, for the same reason, would probably also prove valueless for these species.

The Rose-Bengal and Bromsulphalein clearance tests were also carried out, but preliminary results indicated too rapid clearance of the dyes in normal animals for the tests to be of practical value. The detection of a slight direct positive reaction to the Van den Bergh test was difficult owing to the carotenaemia of bovine plasma and only strong positives have been accepted. Fouchet's reaction has only been recently used by the authors, but positives are absolutely unequivocal and so far have corresponded to Van den Bergh results.

All filter papers used in preparation of protein-free filtrates for N.P.N. were found to give a high blank with Nessler's reagent, and protein-free supernatants have been obtained by centrifugation. A Duboscq type of colorimeter was used for reading absorption in N.P.N. and alkaline phosphatase estimations.

Pathological

All animals examined post-mortem were killed by stunning and severing the neck vessels. Selected tissues were fixed in neutral 10 per cent. formalin-saline and embedded in paraffin, sectioned

at 4 to 5 μ, and stained in each case by haematoxylin and eosin and Mason's haematoxylin-Ponceau-acid fuchsin-light green. In addition, liver sections were treated by Wilder's technique for the demonstration of reticulin. The material for demonstration of fat was usually sectioned within 3 to 4 days of being placed in the fixative; frozen sections were stained by Sudan IV (scarlet red) and counterstained with haematoxylin. Some portions of liver were also fixed in Zenker's fluid and stained as for the formalin-fixed paraffin-embedded material. This procedure seldom offered any advantage.

Liver biopsies were performed by trochar and cannula with the animal recumbent. The site of puncture selected, after preliminary exploration, was about six inches from the mid-line in either of the last two intercostal spaces (Loosmore and Allcroft, 1951). From ten cattle of Boran stock, aged about two years and weighing 350-450 pounds, liver samples have been obtained at weekly intervals without failure. Biopsy material was fixed in 10 per cent. formalin-saline and half was utilised for paraffin, half for frozen sections.

The table shows the animals from which tissues were examined at post-mortem or biopsy, and the time which had elapsed since they were inoculated.

TABLE I

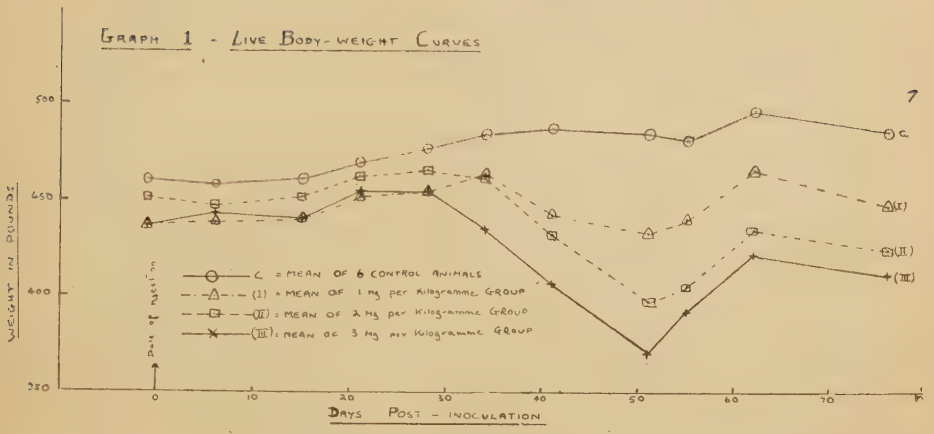
Bovine No.	*Time (in days) since inoculation*	*Tissues Examined*	*Remarks*
924	56	Liver	Biopsy
929	67	Liver	Biopsy
930	67	Liver	Biopsy
	73	Liver	Biopsy
931	48	Liver, kidney, spleen, pancreas, adrenal, thyroid	At death
934	44	Liver, kidney, spleen, adrenal	At death
935	59	Liver	Biopsy
	67	Liver	Biopsy
	73	Liver	Biopsy
936	67	Liver	Biopsy
	73	Liver	Biopsy
937	63	Liver, kidney, spleen, pancreas, thyroid, adrenal pituitary	At death
938	52	Liver, kidney, adrenal, spleen, pancreas, thyroid	At death

RESULTS

Clinical

Skin lesions were not observed but clinical jaundice was detected, at some time or other, in three animals in the 2 mg./Kg. group and five in the 3 mg./Kg. group.

Marked loss of condition was obvious in the higher dosage groups (see Graph I). Grazing deteriorated during the experiment



due to drought, but was sufficient to maintain weight gains in the untreated controls until the last fortnight recorded. The mean bodyweight for groups has been plotted against time (Graph 1). All groups gained weight until day 31 p.i. when the 3 mg./Kg. group started to fall, and on day 35 and day 41 the 2 mg./Kg. and 1 mg./Kg. animals respectively lost weight. The maximum loss of weight was in direct relationship to the dosage and was for 3 mg./Kg., 67 lb.; for 2 mg./Kg., 56 lb.; for 1 mg./Kg., 2 lb., all at day 51. At this time the controls had gained an average of 23 lb. At the last weighing, day 76 p.i., the 1 mg./Kg. group had regained initial weight, but 2 and 3 mg./Kg. groups were still 26 and 25 lb. respectively below the initial weight.

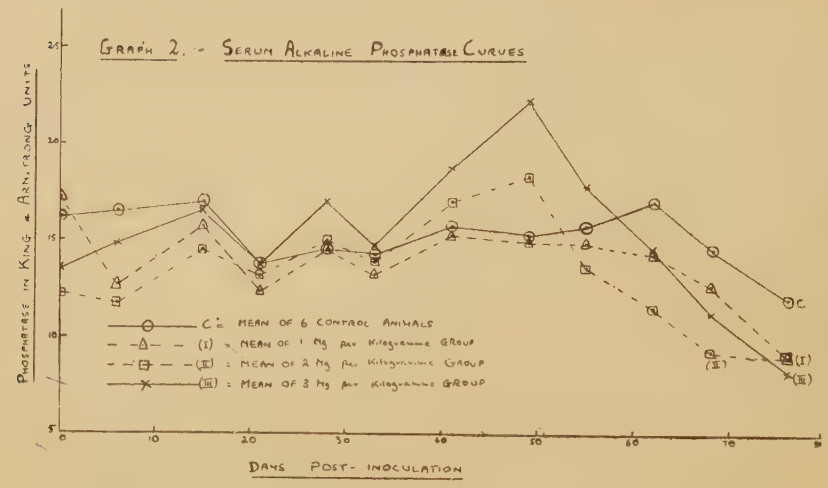
It must be remarked that at no time were lesions or symptoms of photosensitisation observed, in spite of the fact that there were long periods of bright sunlight and no shelter was provided during the whole time of the experiments.

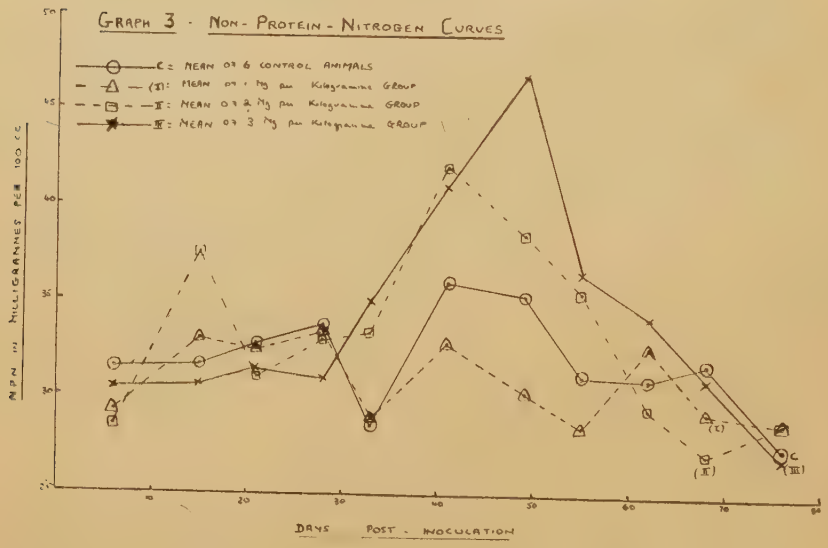
Biochemical

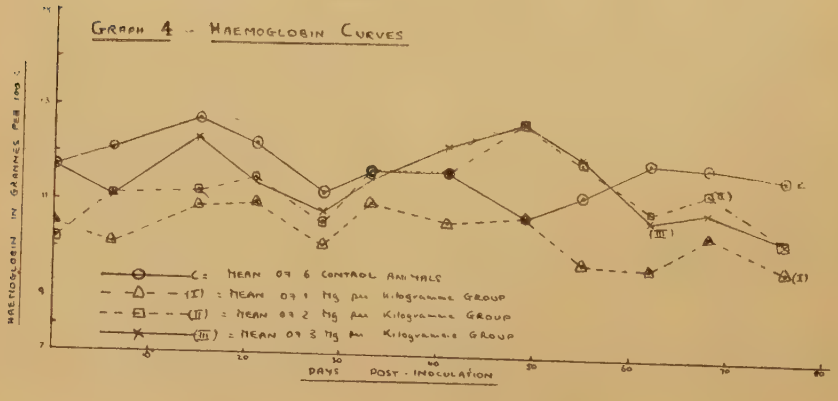
The mean figures resulting from the estimations of the phosphatase, non-protein nitrogen and haemoglobin content of the blood of animals of the various groups have been plotted against time, and are shown in Graphs 2, 3 and 4.*

Phosphatase values for the two higher dosage groups showed a rise commencing just after day 30 (Graph 2). By analogy with the findings reported in jaundice in man (Roberts, 1933), this would point to liver damage of an obstructive type. The figures for one control animal, which after the first few bleedings consistently showed a high phosphatase value, have not been included in the mean for the group. This animal was found at post-mortem ex-

* Full details of the results of these estimations are available at the Veterinary Laboratory, Kabete.


GRAPH 2. - SERUM ALKALINE PHOSPHATASE CURVES
PHOSPHATASE IN KING & ARMSTRONG UNITS
C = MEAN OF 6 CONTROL ANIMALS
(I) = MEAN OF 1 Mg PER Kilogramme GROUP
(II) = MEAN OF 2 Mg PER Kilogramme GROUP
(III) = MEAN OF 3 Mg PER Kilogramme GROUP
DAYS POST-INOCULATION


GRAPH 3 - NON-PROTEIN-NITROGEN CURVES
C = MEAN OF 6 CONTROL ANIMALS
(I) = MEAN OF 1 Mg PER Kilogramme GROUP
(II) = MEAN OF 2 Mg PER Kilogramme GROUP
(III) = MEAN OF 3 Mg PER Kilogramme GROUP
NPN IN MILLIGRAMMES PER 100 C.C.
DAYS POST-INOCULATION


GRAPH 4 - HAEMOGLOBIN CURVES
HAEMOGLOBIN IN GRAMMES PER 100 C.C.
C = MEAN OF 6 CONTROL ANIMALS
(I) = MEAN OF 1 Mg PER Kilogramme GROUP
(II) = MEAN OF 2 Mg PER Kilogramme GROUP
(III) = MEAN OF 3 Mg PER Kilogramme GROUP
DAYS POST-INOCULATION

amination to be suffering from slight chronic inflammation of the ileo-caecal valve and large intestine which may have influenced the result.

Non-protein nitrogen values fluctuated but the means for the 3 mg./Kg. and 2 mg./Kg. groups showed a rise starting at day 31 (Graph 3). This has been assumed to be due to impaired excretory function by the kidneys. Animals receiving 3 mg./Kg. showed the highest N.P.N. figure. Haemoglobin figures for all the groups remained within the normal range and no difference between them was detectable (Graph 4).

Positive Van den Bergh reactions were first seen at day 41, but as bleedings were weekly they might have been demonstrable a little earlier. Four animals in the 3 mg./Kg. group and three in the 2 mg./Kg. group were the first to be noted. Subsequently, all animals in the higher two groups and two in the 1 mg./Kg. group

TABLE II

VAN DEN BERGH REACTIONS

Dose mg/kg.	*Cattle No.*	*Day* 41	43	49	52	56	59	63	65	68	76
1·0	922	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
	923	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
	924	–	– +	+ +	– +	– –	–	– –	– –	– –	– –
	925	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
	926	– –	– –	– –	– –	– +	– –	– –	– –	– –	– –
	927	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
2·0	928	– –	– +	+ +	+ +	+ +	– +	– –	– –	– –	– –
	929	– –	– –	– +	– –	– +	–	– –	+ +	– –	– –
	930	– –	– –	+ +	+ +	+ +	+ +	– –	– –	+ +	– –
	931	+ +	+ +	+ +	Killed						
	932	+ +	+ +	+ +	+ +	+ +	– –	– –	– –	– –	– –
	933	+ +	+ +	– +	– –	– –	– –	+ +	– –	– –	– –
3·0	934	+ +	+ +	Killed							
	935	+ +	+ +	+ +	+ +	+ +	+ +	– –	+ +	+ +	– –
	936	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	– –
	937	– –	+ +	+ +	– +	– –	– –	+ +	Killed		
	938	+ +	+ +	+ +	+ +	Killed					
	939	– –	+ +	– –	– –	– –	– –	– –	– –	– –	– –
Controls	912	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
	913	– –	– –	– –	– –	–	– –	– –	– –	– –	– –
	914	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
	916	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
	919	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –
	921	– –	– –	– –	– –	– –	– –	– –	– –	– –	– –

. The first symbol in every column refers to the direct reaction, the second to the indirect.

were positive at some time. Some were intermittently positive. Reactions tended to start and finish with a positive indirect only. Heifer 936 remained continuously positive for 27 days. All the animals in this experiment were negative at day 76. The results are shown in Table II.

Post-mortem Findings

As previously noted, cases with a marked positive Van den Bergh reaction frequently showed clinical jaundice. Such animals (e.g. Nos. 934, 931, 938) presented varying degrees of generalised icterus at post-mortem examination. The liver sometimes appeared almost normal macroscopically especially when the animal was imperfectly bled out. When the organ was incised, however, to allow escape of the blood, its colour on exposure to the air became more orange-brown and less reddish-brown than the normal. After fixation—especially by formalin—a fine mottling, due to pale areas surrounding the portal tracts, was evident, but this was not well seen through the capsule. Fixed tissue appeared greasy on incision. No marked abnormality was met with elsewhere in the body. The kidneys, in heavily jaundiced animals, were pigmented, especially in the medulla. The spleen was firm, the Malpighian bodies prominent, and in jaundiced animals the capsule was yellowish.

Pathological Changes

The changes observed in the liver of these experimental animals were of the same general type as those already described in cattle suffering from the delayed effects of dimidium bromide treatment (Plowright, Burdin and Thorold, 1952). In this instance, however, it was possible by examination of the various samples of material, obtained by biopsy and after death, to study the probable sequence of changes in the liver resulting from administration of the drug. At about day 40 the cells of the periportal zone showed hydropic degeneration characterised by poor staining of the cytoplasm and in some, though by no means all cases, by signs of nuclear damage; this was accompanied by proliferation and swelling of sinusoidal cells. The sinusoids themselves, however, were congested, particularly near the junction of the normal and degenerate zones. Later, small drops of neutral fat appeared in the hydropic cells and were especially numerous from about day 48 onwards. There was a progressive increase in bile pigment which accumulated particularly in hepatic cells central to the degenerate zones causing them to appear damaged, and in rare instances producing small necrotic foci which became infiltrated with polymorphonuclears and resembled those much more commonly seen in severe cases of photosensitisation caused by the drug (Plowright, Burdin and Thorold, 1952).

From day 59 onwards the size and distribution of the fat globules became increasingly irregular and there was a rapid decrease in the amount of fat demonstrable histologically. By this time signs of regeneration of the parenchyma were well marked, especially in the vicinity of the portal tracts where bi-nucleate cells were abundant.

Frozen sections of kidney material consistently failed to reveal the presence of fatty changes, and no other noteworthy lesion was found which could be associated with the action of dimidium bromide. Bile pigment was conspicuously absent. Paraffin sections occasionally showed areas of cloudy swelling in the convoluted tubules with globular masses of neutrophilic material in the lumen. A few Bowman's capsules had similar globules. Some slight congestion of the glomeruli and medullary vessels was usual. Basophilic concretions, often concentrically laminated and sometimes approaching in diameter that of the collecting tubules, were also found in the medulla. They are common in animals slaughtered at Kabete but are probably not related to the dimidium administration. Nos. 934 and 920 had areas of interstitial nephritis, especially marked in the latter. Dense mononuclear infiltrations, with some early tendency to fibroblast production, characterised this process which again is probably unrelated to the toxicity of dimidium bromide. We have thus been unable to find an adequate histological basis in the kidney for the raised blood non-protein nitrogen figures.

No consistent abnormality was found in the spleen. Animals killed early in the reaction showed some congestion of the red pulp and possibly lymphoid depletion. No excessive accumulation of haemosiderin was observed.

In regard to the adrenal, of four cases examined, two (Nos. 934 and 939) showed pathological changes, these being most marked in the former where they took the form of a dense, interstitial neutrophil and eosinophil leucocyte infiltration of the *zona reticulata* and outer medulla. There was some activation of sinusoidal cells and degenerative changes in the secreting epithelium. In No. 937 a similar though less dense infiltration extended in one part of the gland from the medulla into the *zona fasciculata*. No apparent explanation for these infiltrations was found.

Examination of the thyroid and pituitary revealed no abnormalities.

DISCUSSION

A specific toxic action on the bovine liver due to dimidium bromide at a dosage of 2 and 3 mg./Kg. has been detected and studied. This was followed by a positive Van den Bergh reaction and a rise of serum alkaline phosphatase. The histological findings supported the biochemical results. Toxicity at a dosage of 1 mg./Kg. was demonstrated by a Van den Bergh reaction in two

animals only and histological changes noted in one. On the other hand, cattle which received 1 mg./Kg. at Sotik (Plowright *et al*, 1952), both during the field outbreak and experimentally, were more severely affected. It is obvious that some other factor, nutritional or climatic, played a part in determining the degree of liver damage and the onset of clinical photosensitisation.

If liver lesions are considered as a separate entity, it will be seen that they were demonstrated constantly in animals dosed at all levels above 1 mg./Kg. and doubtless were fundamental in the production of the clinical toxicity.

The occurrence of severe congestion and even haemorrhage in the periportal zone, together with the sparing of the central part of the lobules, did not seem to accord easily with the usual theories of liver injury and its relation to the intralobular circulation (Himsworth, 1950). The absence of vascular lesions, and of any considerable coagulative necrosis differentiated the condition from the periportal lesion which has been regarded as characteristic, to some extent, of human eclampsia (Govan and Mukherjee, 1950). It seemed reasonable to assume that the circulation to the central part of the lobules was adequate in spite of very severe fatty infiltration in the periphery. The sinusoids were definitely dilated periportally, not constricted and the portal vessels were not engorged. On the other hand, the accumulation of bile pigment, especially in the midzone, pointed to an interference with its normal excretion via the biliary canaliculi to the bile ducts.

The sudden onset of a degenerative process in the liver after a somewhat prolonged "latent" period recalls the massive necrosis produced in rats on deficient diets and preventable by cystine or tocopherol supplements. The time-lag, too, is reminiscent of that in Chastek paralysis (Yudkin, 1949) where after a month of raw fish-feeding animals rapidly develop paralysis and die in 1 to 4 days after onset of symptoms. "Bracken poisoning" also is reported to occur only after 2 to 4 months of continuous ingestion of the plant (Evans and Evans, 1949). The pathology of the liver injury in delayed dimidium bromide poisoning is reminiscent of the fatty infiltration produced in rats on diets deficient in lipotropic factors (choline, methionine, possibly inositol, riboflavin and pyridoxine). These lesions, on the contrary, are recorded as being of much slower development and to lead, if prolonged, to diffuse hepatic fibrosis. A single dose of 2 to 3 mg./Kg. of dimidium bromide certainly induced some increase in portal tract and periportal reticular fibres, and in the former situation was followed, in some cases, by a slight collagenous increase with bile duct proliferation. In general, however, the residual lesion was of small extent and parenchymal regeneration was complete. Knowledge of the effects of simple vitamin deficiencies in the bovine is scanty. There is evidence for the microbial synthesis in the rumen, and subsequent utilisation by the host, of vitamin B_1, biotin, folic acid, nicotinic

FIG. I.

FIG. 1. Bovine 938: 3 mg./Kg. dimidium bromide subcutaneously. Killed day 52 p.i. Liver—paraffin section, Masson's trichrome stain. The portal tracts are surrounded by pale staining areas with degenerating vacuolated cells. (× 100.)

FIG. II.

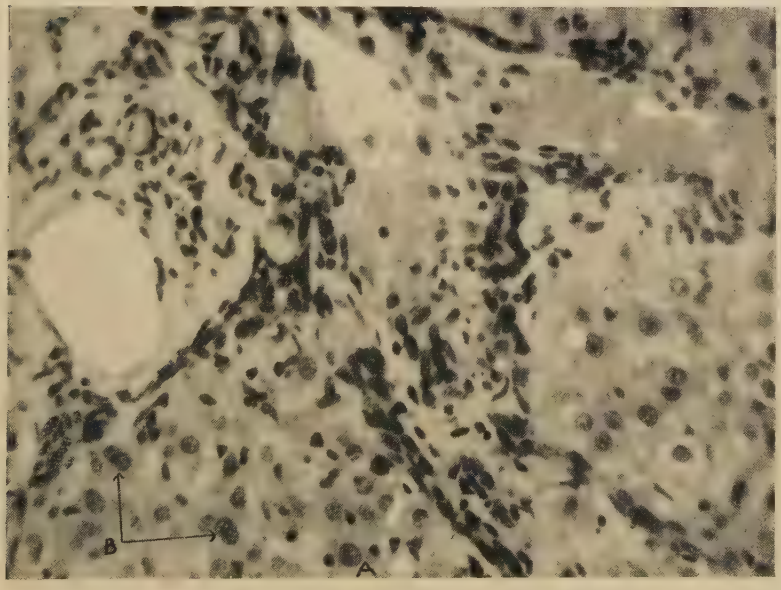


FIG. 2. Bovine 935 : 3 mg./Kg. dimidium bromide intravenous. Liver biopsy, day 67 p.i. Paraffin section : H. & E. Portal tract showing fibrosis, bile duct proliferation. See also (low centre) large hyperchromatic nucleus (A) and (low left) numerous binucleate cells (B). × 400.

acid, pantothenic acid, vitamin B_6 and riboflavin (Kon and Porter, 1947). There is thus no pathological observation with which to compare these findings.

Chemically, it appears that inhibition of three essential metabolites, choline, nicotinic acid and riboflavin, is possible by virtue of the similarities of structure. The mode of degradation and of detoxification *in vivo* of dimidium bromide is not known.

It is probable that the important parts of the dimidium bromide molecule are: (1) the aromatic amino groups, capable of combining with protein molecules by hydrogen-bonding or condensation with carboxyl groups and (2) the hetero-aromatic quaternary group, capable of reversible reduction, and apparently essential for trypanocidal activity in this class of compounds (Brownlee, Goss, Goodwin, Woodbine and Walls, 1950).

The possession of (1) could explain the retention of the drug in the body for the requisite time. Group (2) might interfere in the biological methylation of choline or methionine, or with other functions of choline by competitive inhibition. It might also inhibit the action of Coenzyme I or Flavine-adenine-denucleotide by its easily reversible oxidation-reduction properties. It is not feasible with the facilities at Kabete to explore these possibilities by microbiological methods or even to work out the stereochemical considerations necessary. It is hoped to throw some light on this theory, however, by attempting prevention of the liver lesions by vitamin supplementation.

SUMMARY

The administration of dimidium bromide in a dosage of 2 or 3 mg./Kg. to normal Zebu-type cattle regularly produced liver dysfunction.

Biochemical evidence of this dysfunction was provided by the appearance of a direct Van den Bergh reaction at about day 40. Weight records together with serial estimations of non-protein nitrogen and alkaline phosphatase indicated a disturbance beginning at about the same time.

The dysfunction was associated with periportal degeneration of the liver, at first largely of a hydropic type, but rapidly becoming fatty. Residual lesions were slight.

The possible relationship to conditioned deficiencies of essential metabolites is discussed.

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INVESTIGATIONS INTO DELAYED TOXICITY DUE TO DIMIDIUM BROMIDE

IV. Effect of High Dosage Levels of Dimidium Bromide in Cattle

By

W. Plowright and M. L. Burdin

Veterinary Research Laboratory, Kabete, Kenya.

INTRODUCTION

In a previous communication (Burdin and Plowright, 1952) the biochemical and histological effects of dosage with dimidium bromide in the region of the therapeutic level, were studied. It was considered desirable, however, to investigate what happens in cattle receiving much higher doses.

In a pilot experiment using eight low grade cattle given dimidium bromide intramuscularly in doses ranging from 8·8 to 22 mg./Kg. all the animals died between days 36 to 43 post inoculation. The changes at post mortem seemed to differ from those already reported (Thorold and Plowright, 1952) in the livers of animals showing typical delayed toxicity, as the fatty infiltration affected the whole of the lobules. In addition, the heart commonly showed large ecchymoses in the left ventricle and a very pale myocardium, lesions which had not previously been observed.

Two further experiments were therefore arranged with the object of (*a*) determining the effects of a single large dose of the drug subcutaneously, (*b*) investigating if small doses, repeated at short intervals, would each produce a series of reactions after the usual "latent" period, the summation of these reactions causing possibly a massive liver lesion; or alternatively if the effect would be of the type produced by a single large dose.

SINGLE LARGE DOSES

Six Boran heifers were injected subcutaneously behind the shoulders with a 2 per cent. solution of dimidium bromide, batch 80. Two were given 10 mg./Kg., two 15 mg./Kg. and the remaining two 20 mg./Kg. The cattle were allowed to graze and weighed at first fortnightly, then weekly (together with six uninoculated controls).

The fate of the six injected animals is summarised in the following table: —

TABLE I

Group	*Animal No.*	*Dose*	*Clinical Findings*
I	917	10mg./Kg.	Died day 21 of intercurrent infection.
	915	„	Loss of weight with maximum (50 lbs.) at day 51.
II ...	920	15mg./Kg.	Rapid loss of weight (165 lbs. at death on day 50). Clinical jaundice from day 44.
	918	„	Loss of weight (185 lbs. at day 50). Jaundice from day 40.
III	911	20mg./Kg.	Died day 13. Cause undetermined.
	910	„	Died day 44, with weight loss of 114 lbs.

Biochemical Observations.

In Group I no observations of significance were observed. In Group II there was a positive direct van den Bergh reaction at day 44 and subsequently, with elevated serum alkaline phosphatase and a temporary rise in N.P.N. No. 918 was van den Bergh positive until day 105. Regular examinations were only made after jaundice developed. In Group III the phosphatase and N.P.N. were high at day 35 in 910. The van den Bergh reaction was negative on same day.

Pathology.

Group I. In 917 there was necrosis of muscle underlying the site of inoculation.

Group II. In 920 there was congestion, haemorrhage and small droplet fatty infiltration of a wide, well defined periportal zone. A reticulo-endothelial reaction and pigment accumulations were also present (Plowright *et al*, 1952). No significant changes were seen elsewhere apart from necrosis of large areas of muscle underlying the site of inoculation.

No. 918, destroyed on day 113, showed the presence of bile pigment in the liver, together with bile duct proliferation.

Group III. No. 910 showed an infiltration of fat globules of varying size and intensity of coloration affecting mainly the centres of the liver lobules. There was also much fat in the epithelium of collecting tubules of the kidney and a smaller quantity in the convoluted tubules.

REPEATED SMALL DOSES INTRAVENOUSLY

Ten Boran heifers were divided into five groups and received the following treatment: —

TABLE II

Group	*Animal No.*	*Dosage*	*Total*
IV	1501 1497	Uninoculated controls.	—
V	1498 1502	1·0mg./Kg. fortnightly intravenously... ...	3mg./Kg.
VI	1495 1500	0·5mg./Kg. bi-weekly intravenously	6mg./Kg.
VII	1496 1499	2·0mg./Kg. fortnightly intravenously... ...	6mg./Kg.
VIII	1503 1504	1·0mg./Kg. bi-weekly intravenously	11mg./Kg.

All ten were bled bi-weekly and immediately before inoculation. The first injections were carried out on 4th April, 1951, and at the stated intervals thereafter until 14th May when three animals had died. The total doses received are given in the table. In this experiment days post inoculation refers to days following first inoculation.

Groups VI and VIII lost weight and condition rapidly, whilst the loss of weight in groups V and VII was more slow at first. At day 38, 1499 and 1503 were found comatose and were killed. These were followed by 1504 on day 40, 1500 on day 45, 1495 on day 48, and 1496 on day 63. All showed the same syndrome of sudden prostration and coma. No skin lesions suggestive of photosensitisation were observed.

Biochemical Observations.

Haemoglobin remained normal for all animals. No. 1496 showed high phosphatase values commencing on day 40 and reached about 20 units by day 47. No. 1499 was high a week before death and 1504 on the day of death. All the others remained steady. A van den Bergh reaction of the delayed direct type was first recorded in 1499 on day 33. Three more animals, 1504, 1498 and 1502, were positive (delayed direct) on day 40. The first positive immediate direct reactions were seen in sera from 1495 and 1496 on day 47, and Fouchet's reaction, used for the first time, confirmed the presence of hyperbilirubinaemia.

Postmortem Findings.

Six animals came to postmortem examination (Table III). Slight degrees of jaundice, especially noticeable in the renal medulla, were seen in 1499 and 1504. In 1496, 1504, 1499 and 1500 the liver, on removal from the body, became an orange-brown colour on fixation, and that of 1500 showed a distinct regular

mottling. Marked friability and swelling were not observed. In the case of 1496 the liver had a shrunken appearance, especially in the inferior part, and was tough to cut. The serosal and cut surfaces showed a fine regular fibrosis. This is the sole instance where a distinct atrophy and portal cirrhosis has been seen following the administration of dimidium bromide.

In three animals which died during the rapid type of reaction (1503, 1504 and 1500), irregular blackened haemorrhages and erosions were noted at the pyloric end of the fourth stomach, and along the edges of the mucosal folds. In 1500 this lesion was accompanied by an extensive oedematous infiltration of the submucosa. Nos. 1499, 1503, 1504 and 1500 all showed a congestion of the medulla of the carcase lymphatic glands.

No other macroscopic changes were present.

Table III

Serial Liver Biopsies.

Day	*1*		*9*		*16*		*23*		*36*		*44*		*58*		*64*	*70*	*77*
Bov. No.	*FS*	*PA*	*FS*	*AC*	*FS*	*PA*	*FS*	*AC*	*FS*	*PA*	*FS*	*PA*	*FS*	*PAF*	*FS*	*FS*	*FS*
1495	+	+	+	+	+	+	+	+	+	+	+	+	D(48)				
1496	+	+	+	+	+	+	+	+	+	+	+	+	+	+	(D63)		
1497	+	+	+	+	+	+	+	+	+	+	+	+	—	—	+	+	+
1498	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1499	+	+	+	+	+	+	+	+	+	+	D(38)						
1500	+	+	+	+	+	+	+	+	+	+	+	+	D(45)				
1501	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
1502	+	+	+	+	+	+	+	+	+	+	+	+	+	—	+	+	+
1503	+	+	+	+	+	+	+	+	+	+	D(38)						
1504	+	+	+	+	+	+	+	+	+	+	D(40)						

\+ = successful biopsy.
FS = fixed in 10 per cent. formol-saline.
PA = fixed in saturated alcoholic picric acid for glycogen demonstration.
AC = fixed in acetone, for alkaline phosphatase demonstration.
PAF = fixed in alcoholic picric acid—formalin, for glycogen demonstration.
D = dead or destroyed. Figures in brackets refer to day *p.i.* of death or destruction.

Histological Technique.

Liver biopsies were performed on all animals at the intervals stated in Table III. The biopsy technique has been described previously (Burdin and Plowright, 1952), cores obtained being divided into approximately 1 cm. lengths to put into the various fixatives. It will be seen that no failure to obtain tissue occurred in over 70 consecutive attempts. The material obtained sometimes consisted partly or wholly of scar tissue formed in the liver at the site of previous punctures. This was usually discarded and another attempt made with the cannula inserted in a slightly different direction. Some animals (e.g. 1502, 1498) were subjected to liver biopsy on ten occasions, entries in each case being in a

slightly different part of the last two intercostal spaces at a level of about six inches from the midline. No untoward results were observed, in spite of the failure to observe strict precautions against infection.

Where a postmortem examination has been possible, no major macroscopic pathological differences have been noted between different lobes of affected livers; and, in the few instances examined, microscopical findings have indicated that no differences were present.

Tissues removed at postmortem examination have been treated as described in previous communications (Plowright *et al*, 1952; Burdin and Plowright, 1952). On two occasions attempts to demonstrate alkaline phosphatase in sections were unsatisfactory. The method used was the revised Gomori technique described by Glick (1949). Glycogen was demonstrated by the Bauer-Feulgen method as described by Cowdry (1948). It was difficult to obtain uniformly good fixation of glycogen, even when the small cores of tissue (3 to 4 mm. diam.), obtained with the biopsy cannula, were placed in a large volume of fixative. Biopsy tissue was examined as paraffin sections, stained with haematoxylin and eosin, on three days only (1, 9 and 23).

Histology.

Group IV. In the uninoculated controls (1501, 1407) fat globules, strictly limited to the von Küpfer cells, could be found in the liver throughout the course of investigation. Glycogen in abundance could always be demonstrated, especially centrilobularly. This finding was of particular interest on days 36 and 44, when glycogen had almost completely disappeared from the inoculated animals.

Group V. In 1498 and 1502 glycogen was greatly reduced in amount on day 36, and some scattered liver cells also showed numerous large vacuoles in which no fat could be demonstrated. A narrow zone of periportal degeneration was present in both animals on day 44 but fat globules had disappeared on days 58 and 70 respectively. Bile pigment was demonstrable in macrophages up to day 70 and mild proliferation of bile ducts occurred.

In 1495 and 1496 glycogen was much reduced on day 36. A periportal zonal degeneration beginning by day 36 was seen in both animals. In 1495 only a narrow zone around the central vein was spared. Large vacuolated cells, apparently free from fat, as described in Group V, were present especially in 1496. Bile duct proliferation and portal fibrosis was marked at slaughter on day 52 in 1496. Globular homogeneous masses of an eosinophilic material were found in Bowman's capsule and the kidney tubules of 1495.

Nos. 1503, 1504, 1499, 1500 died between days 38 and 45 and showed similar lesions; i.e. reduction in glycogen on day 36 with appearance of fat throughout the lobules except in the case of 1500, where it tended to be restricted to the central zone. Hepatic cells were pale and swollen with shrinkage and crenation of many of the nuclei and compression of the sinusoids. There was no bile duct proliferation.

The kidneys showed some tubular degeneration with the presence of globular masses in the lumen as in the preceding group. In 1500 the quantity within Bowman's capsule was often sufficiently great to cause compression of the glomerulus. The liver and kidney changes increased in severity in the order given above; i.e. 1503, 1504, 1499, 1500.

SUMMARY

The delayed reaction which follows the administration of dimidium bromide to cattle can take one or two forms which clinically, biochemically and pathologically are quite distinct. Both have their onset, as determined by serial biopsies, at some time about the end of the fifth week following inoculation, and are characterised by fatty infiltration of the liver.

The type of reaction does not seem to depend solely on the dosage; animals which have received up to 3 mg./Kg. have, in our experience, consistently showed the more prolonged type with positive direct van den Bergh reactions, elevated serum alkaline phosphatase and a well defined periportal lesion.

Above this level results are inconsistent. For example, 920, which received 15 mg./Kg., showed this kind of response but two of four animals, which received a total of 6 mg./Kg. over a period of six weeks, developed a diffuse liver lesion resembling that seen in animals at a higher dosage level.

The second type of reaction is accompanied by no rise in serum alkaline phosphatase and the van den Berg reaction, if positive, is a delayed direct one, indicative of toxic rather than obstructive jaundice. Animals with this type of reaction are usually dead or *in extremis* by about the 45th day and often show a terminal coma; on the other hand, where the lesion is periportal and a positive direct van den Berg reaction may develop, the animal commonly survives until at least the 48th to 50th days and may even recover.

The effect of small doses of dimidium bromide, administered intravenously at frequent intervals, is the same as that of a single large dose. There is no reduction in the "latent" period and, except in one case (1946), the lesions were identical with those caused by a single massive dose. In 1496 there was evidence of a commencing fine portal cirrhosis.

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STUDIES ON EXPERIMENTAL STREPTOCOCCAL MASTITIS

VII. Immunological Studies In Goats

By

H. H. Holman, I. H. Pattison and W. S. Gordon

Agricultural Research Council, Field Station, Compton, Berkshire.

INTRODUCTION

In the first paper of this series attention was called to an increase in mouse protection provided by the sera from goats affected with experimental mastitis due to intramammary inoculation of *Str. agalactiae* strain S.13.

The present paper records the production of this mouse-protecting antibody and of agglutinins in goats vaccinated with living cultures in broth of S.13 and the successful protection of these goats against intramammary infection with this same organism.

Experimental results are presented under four headings: —

1. Clinical reactions and changes in leucocyte counts caused by vaccination.
2. Production of detectable antibodies.
3. Protection against experimental mastitis by different methods of immunisation.
4. Observations on crises in streptococcal mastitis.

MATERIALS AND METHODS

The husbandry of the goats used and the general bacteriological and haematological methods employed have been described (Pattison, Taylor and Holman, 1950). The method of making the neutrophil count (Breed smear) in milk was detailed by Pattison and Holman (1951).

Tests for the detection of antibody

Passive protection test in mice. Reference has previously been made to the methods used (Pattison, *et al.,* 1950; Holman, Pattison and Taylor, 1950). Freshly-drawn serum was passed through a Seitz EK sterilising pad and a dose of 0·5 ml. was inoculated intraperitoneally into each of ten mice; this was followed after 18 hours by an intraperitoneal inoculation of 0·5 ml. of an 18-hour Todd-Hewitt broth culture of S.13. Deaths within 24 hours were recorded.

With non-immune serum the death rate in mice was 95 per cent., and even the most potent serum failed to protect all mice over a series of tests. The reliability of the test can be ascertained from the following data: —

(*a*) The distribution of the results for 200 tests, each of ten mice, on serum that did not contain antibody was as follows: —

Number of mice killed in 24 hours ...	5	6	7	8	9	10
Distribution of 200 tests	0	2	5	24	62	107

This shows that a death rate of six mice out of ten occurred only once per 100 tests, and the sample is sufficiently large to permit the general conclusion that the survival of four out of ten mice indicates the presence of some antibody in the serum under test.

(*b*) Six samples of antibody-containing serum taken at the same time from the same goat were filtered separately and tested for antibody. This was done for four goats, and gave the following results: —

SIX SERUM SAMPLES FROM EACH OF FOUR GOATS

Goat	*1st sample*	*2nd sample*	*3rd sample*	*4th sample*	*5th sample*	*6th sample*
A	3	1	4	4	5	5
B	2	3	3	2	2	5
C	1	3	4	3	2	2
D	2	2	2	1	2	5

Figures give number of deaths out of ten mice at the 24th hour.

The variance of these figures shows that there must be a difference of four mice dead between tests of any two sera to indicate a significant difference in antibody. Confidence levels for comparing two tests are: —

Difference in mice dead out of ten	*p*
4	0·05/0·02
5	0·02/0·01
6	0·01/0·001

Agglutination test. A plate test modelled on the rapid agglutination test for *Br. abortus* (Huddleson, 1934) was used. A thick antigen was made by growing strain S.13 in several litres of Todd-Hewitt broth for about twenty hours at 37° C. The culture was then heated to 60° C. for one hour, filtered through several layers of gauze and centrifuged. The residue, representing about one-

twentieth of the original bulk, was standardised to the titre of a standard serum by the addition of supernatant fluid; 0·5 per cent. phenol was then added as a preservative. Dilutions of serum with saline were made in the series 1 : 1, 1 : 2, 1 : 4 etc., and drops of 0·02 ml. were mixed with antigen and read over a viewing box after two minutes. Goats that had not been infected with S.13 were invariably negative at 1 : 1.

Intramammary test dose used to examine the efficiency of vaccination

S.13 suspended in 10 ml. of sterile Todd-Hewitt broth was inoculated into one side of the udder. This dose was calculated to contain 1,000 organisms, but plate counts indicated that the number of living organisms inoculated varied from 1,000 to 25,000 in different experiments.

Vaccine. This consisted of *Str. agalactiae* strain S.13 grown for 18 hours in Todd-Hewitt broth.

Presentation of data in Table II

Milk yield. Readings for the first two or three days after inoculation were confused by the inevitable non-specific reaction to the introduction of foreign material into the udder, and in some control animals a compensatory reaction, shown histologically as the activation of previously non-functional tissue, affected the milk yield after about the fourteenth day. In this Table, therefore, comparisons between vaccinated and control animals were limited to the first fortnight after inoculation of the test dose with particular emphasis on the differences noted in the second week.

A fall in milk yield, suggesting impairment of function, was regarded as the most important measurement. The natural proportion of the yield of one side of the udder to the other was first observed over the week prior to infection with the test dose. The method of estimating the loss due to infection was to record the yield of the infected side as a proportion of that of the healthy side. If the totals for the pre-inoculation week were called "a" for the side to be inoculated, and "b" for the control side, then a factor F was introduced so that $\frac{a}{b} \times F = 100$ per cent. This factor was obtained from $\frac{b}{a} \times 100$. After the test dose the total yield for a seven-day period was recorded as $\frac{a}{b} \times F$.

Neutrophil (Breed smear) count in milk. Neutrophil counts in millions per ml. of milk are recorded as the geometric mean of seven consecutive daily counts.

Counts of strain S.13 in milk. Streptococci in milk are in thousands per ml. Counts were made by spreading a 0·02 ml. drop of milk over the surface of one Bactotryptose, ox-blood agar plate and counting colonies after 24 hours' incubation at 37° C. The large number of samples being handled made accurate counts by dilution impracticable, so that certain approximations were neces-

sary. Confluent growth over the plate was called " 2,000 " colonies; counts over 500 but without confluent growth were called " 750 "; counts between 200 and 500 were approximated to the nearest 20; actual counts were made up to 200.

RESULTS

Clinical Reactions and Changes in Leucocyte Counts Caused by Vaccination

Intradermal, subcutaneous, intraperitoneal, and intravenous routes of inoculation were used.

Intradermal and subcutaneous inoculation caused transient swelling at the site of inoculation, but no abscess formation; after a few days only a small firm nodule was detectable. Sometimes a systemic disturbance, characterised by dullness, inappetence and some fall in milk yield, was noted. Intraperitoneal inoculations were followed by a systemic disturbance lasting some 24 hours. Intravenous inoculations produced systemic disturbance and lameness; in three of five goats inoculated by this method the lameness was permanent.

When lactating animals were inoculated with S.13 by any method, there was often a rise in the neutrophil count in the milk. This rise appeared within 24 hours of inoculation and had usually disappeared by the 48th hour. It varied in extent from animal to animal, and in the same animal from inoculation to inoculation; the count from different sides of the same udder was often quite different. In some cases the rise was very marked, e.g. in one case over 12 millions per ml.

Large numbers of milk samples were cultured but *Str. agalactiae* was never detected during immunisation, even when numerous intradermal inoculations were made over the udder itself.

Counts of blood leucocytes made on four animals showed that each vaccination was followed by a fall for one or two hours after inoculation which was usually of one or two thousand per c.mm.; only on one occasion did the total count fall as low as three thousand per c.mm.

Production of Detectable Antibodies

Production of antibody following infection of the udder

The production of mouse-protecting antibody and of agglutinins following inoculation of S.13 into one side of the udder, as determined by thrice-weekly tests, is shown diagrammatically in Fig. 1. Goat 1 in this Figure is an example of the quick production of antibodies. Mouse-protecting antibody and agglutinins were present in this animal at the beginning of the second week, and after the fourth week there was an average of eight mice alive, while the agglutination titre was 1 : 256 or more. In goat 2 the appearance of antibodies was delayed, so that agglutinins appeared

FIG. 1

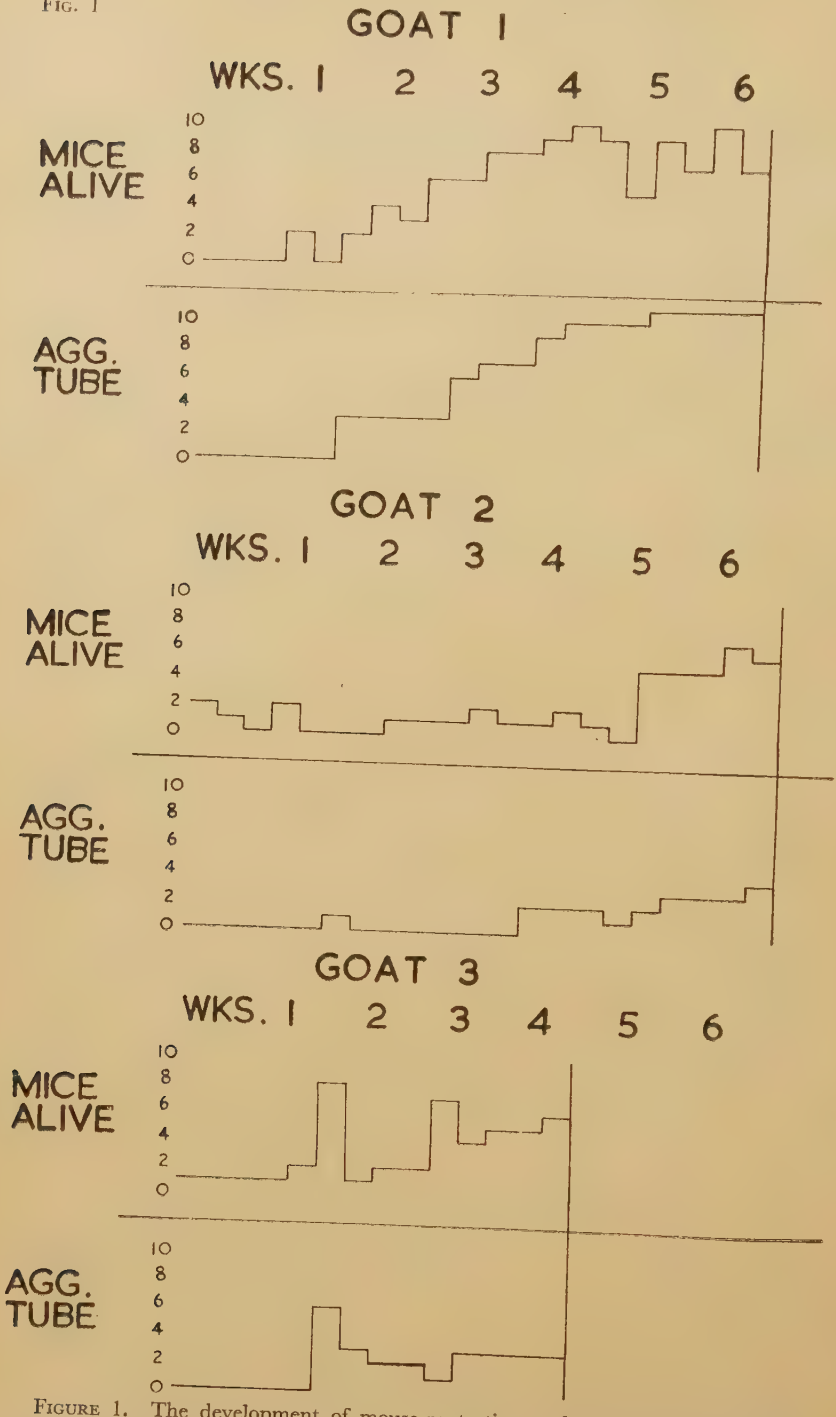

FIGURE 1. The development of mouse-protecting and agglutinating antibody in the blood serum of three selected goats inoculated into one side of the udder with *Str. agalacitae* strain S.13.

Goat 1—good and fairly rapid antibody formation.
Goat 2—poor and delayed antibody formation.
Goat 3—early formation of antibody, its disappearance, and subsequent reappearance.

AGG. TUBE refers to the number of the dilution in the plate method, 1, 2, 3, etc. referring to 1 : 1, 1 : 2, 1 : 4, etc.

after the fourth week and mouse protection after the fifth week. Goat 3 is included to show a phenomenon noted on several occasions, namely the detection of a fairly high level of antibody on one occasion early in the disease, followed by a fall in titre, and then by an irregular recovery a week or so later. There was a little evidence to suggest that this transitory rise in antibody might be connected with a "crisis" (q.v.) of the disease.

The variation in time before mouse-protecting antibody could be detected in the serum is emphasised in Table I, which gives the results of repeated tests on twenty-five goats subsequent to infection of the mammary gland. The longest period was in one goat which showed a single positive test in twenty-seven weeks, followed by negative tests until observations were stopped five weeks later.

TABLE I

REPEATED MOUSE PASSIVE IMMUNITY TESTS FOR THE PRESENCE OF STREPTOCOCCAL ANTIBODY IN THE SERA OF 25 GOATS EXPERIMENTALLY INFECTED WITH STREPTOCOCCAL MASTITIS.

Group	*Number of animals*	*Serum first positive : weeks after infection*											
		2	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*over 12*
Serum first tested within two weeks of infection	10	4	2	—	2	—	—	—	—	—	—	—	2
Serum first tested 3–4 weeks after infection	15		4	3	1	1	—	—	—	2	1	—	3

As the inoculated organism persisted in the udder of the majority of animals there was little opportunity to measure the persistence of antibody following freedom from infection, but it can be said that two animals that had freed themselves from infection at four months became negative to the mouse test at six months.

Serum agglutinins were not present in non-infected goats, but before the end of the second week after infection they were usually detectable at a titre of 1 : 1 or 1 : 2. Thereafter the titre rose gradually to reach a maximum, in exceptional cases, of 1 : 1024. When the serum titre was 1 : 8 some agglutinins were detectable in the milk whey from both inoculated and non-inoculated sides of the udder, the inoculated side usually having the higher titre. Exceptionally the whey titre was as high as that of the serum, varying from 1 : 4 early in the disease to 1 : 32 at a later stage. The highest titre recorded in whey was 1 : 64.

Production of antibody by vaccination

To find an inoculation technique that might be suitable for producing a high level of antibody, goats were inoculated intraperitoneally with living cultures of S.13 in Todd-Hewitt broth at intervals of one week.

The dose for each goat was the same at each inoculation, but varied in goats in a series from 0·3 ml. to 300 ml. The amounts of antibody detected by the mouse test for the two extreme doses and the mean dose (10 ml.) in this series are depicted in Fig. 2. For the first three weeks, each inoculation lowered the death rate in the succeeding week, after which it varied within the limits of accuracy of the test. Concomitant agglutination tests ranged over four dilutions, e.g. from 1 : 32 to 1 : 256 in the goat receiving the 10 ml. dose.

FIG. 2

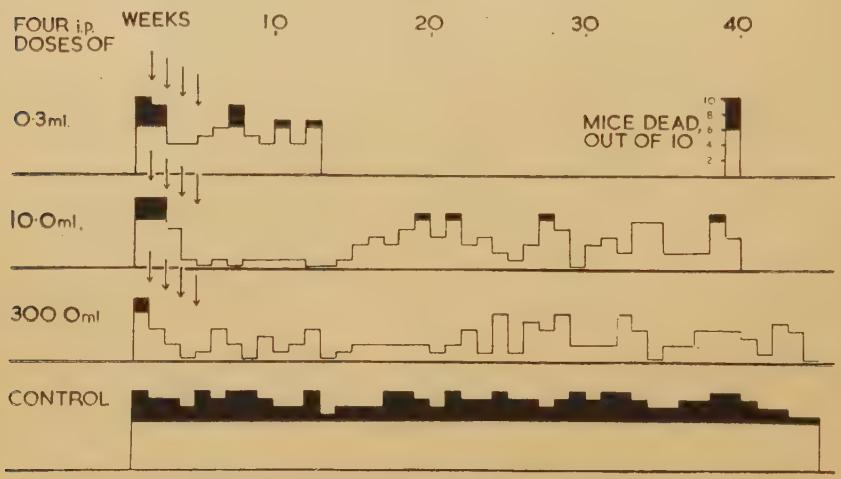

FIGURE 2. The development of mouse-protecting antibody in the blood serum of three selected goats inoculated intraperitoneally four times at weekly intervals with different doses of living whole broth culture of *Str. agalactiae* strain S.13. The four arrows indicate four weekly inoculations of S.13. Black areas of the columns are for visual contrast and indicate more than six out of ten mice dying in any one test.

The conclusion drawn from these experiments was that a dose of 10 ml. of living culture given intraperitoneally each week for four weeks produced a reasonable level of antibody that could persist for at least eight months. The first experiments on protection were carried out using this immunising routine.

It can be seen from Fig. 2 that in the case of the animal immunised by the 300 ml. doses, detectable antibody was still present when observations finished at 45 weeks. In view of this, weekly testing of two goats previously referred to by Holman, Pattison and Taylor (1950), one immunised by the intraperitoneal method and the other by the intravenous method, was continued.

The results of these weekly mouse tests up to a total period of 132 weeks are given in Fig. 3. If allowance is made for the limited accuracy of the test, it will be seen that the level of antibody remained approximately the same for more than two-and-a-half years.

FIG. 3

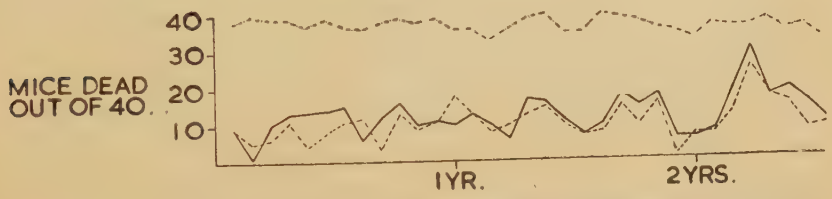

FIGURE 3. The persistence of mouse-protecting antibody in the blood serum of two goats examined every week for 132 weeks. Weekly tests of 10 mice each are summarised in four-weekly periods.

Serum from :—
—·—·—·—·— non-immune control goat.
———————— goat prepared by intraperitoneal inoculation.
— — — — — goat prepared by intravenous inoculation.

Correlation between mouse-protecting antibody and agglutinating antibody

In Fig. 1 there is a clear association between the formation of mouse-protecting and agglutinating antibodies, but when individual tests are compared this association is by no means obvious. The limited accuracy of the mouse test must, of course, obscure the relationships to some extent, but where sufficient numbers of concomitant tests are available some correlation is apparent. This is shown in the following Table which records the results for 179 tests on blood serum: —

Agglutination titre	*Deaths out of ten mice*		
	0–4	5–7	8–10
0	0	0	42
1 : 1 to 1 : 8	6	10	19
1 : 16 to 1 : 128	60	18	4
1 : 256 to 1 : 1024	15	5	0

Protection against Experimental Mastitis given by different Methods of Immunisation

Protection by vaccination

Note on variations in the individual reaction of goats. In a previous paper (Pattison and Holman, 1951), the types of reaction

to intramammary inoculation of strain S.13 were classified as marked, intermediate, or minimal. The milk of marked reactors was greatly reduced, but there was little loss in the case of minimal reactors.

This variation, and the impossibility of forecasting the type of reaction in any group of individuals, created a difficulty in the adequate control of vaccination experiments. For example, in an experiment involving exposure to a test dose of, say, four vaccinated individuals and two controls, the two controls might both be marked reactors, and by comparison the vaccinated individuals might appear well protected. In another experiment, by contrast, the two controls might be minimal reactors, so that the resistance of the vaccinated group might appear slight. Similar individual resistance could be postulated among vaccinated animals. The obvious method of avoiding this difficulty was to increase the number of animals in all experiments, but for practical reasons this was not possible.

The importance of individual susceptibility or resistance

FIG. 4

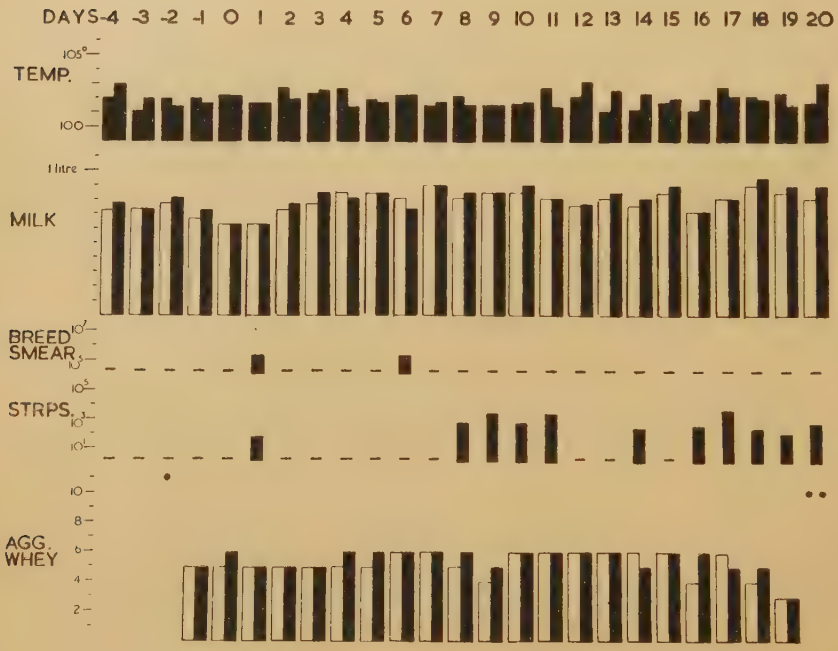

FIGURE 4. Measurements made on one immunised goat (second goat in Group 3, Table II) Day " O "=day of inoculation. The black columns under " Milk " and " Agg. whey "give values for the inoculated side of the udder and the white columns for the non-inoculated side.

" Breed smear " records count of neutrophils in milk.

*Blood serum agglutination titre before inoculation—1 : 512.

**Blood serum agglutination titre twenty days after inoculation—1 : 512.

among animals became more obvious at each experiment, so that finally it was considered impossible to compare one small experimental group with another for the purpose of comparing different methods of immunisation. It was therefore decided to neglect differences in the methods of immunisation and to deal with all goats as belonging to one of two groups, either immunised or control. In view of this decision the different immunising routines are recorded only in Table II and are not particularised in the text.

General results. The protection afforded by vaccination is most clearly seen by comparing the daily records for a vaccinated goat showing average protection (Fig. 4) with a control showing a marked reaction (Fig. 5).

FIG. 5

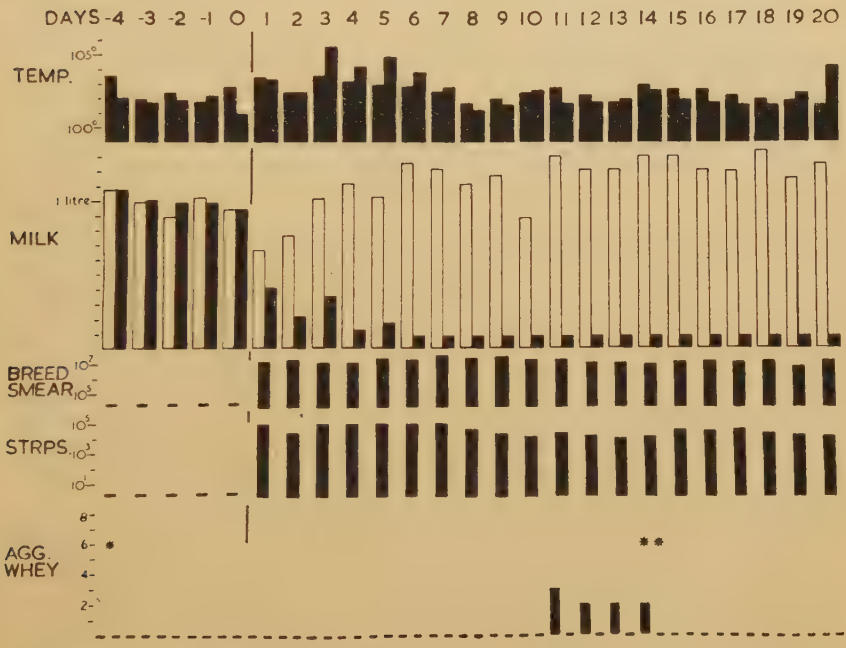

FIGURE 5. Measurements made on one control goat (third control in Table II). Day "O" = day of inoculation. The black columns under "Milk" and "Agg. whey" give values for the inoculated side of the udder and the white columns for the non-inoculated side.

"Breed smear" records count of neutrophils in milk.

*Blood serum agglutination titre before inoculation—nil.

**Blood serum agglutination titre fourteen days after inoculation — 1 : 64.

The body temperature, recorded twice daily, shows a reaction during the first week in the control goat, but none in the immunised goat. The milk yield shows a great fall in the inoculated side of the control goat, while in the immunised animal the gland continues to secrete at preinoculation level. The neutrophil count

in milk (recorded as " Breed smear ") is continuously over a million per ml. in the control animal, but only two isolated counts, both below a million, are seen in the immunised goat. Streptococci are present in the milk in the control goat in numbers that were innumerable without dilution, but which have, for convenience, been recorded as in the neighbourhood of 100,000 per ml.; in contrast, organisms were only detected in the immunised goat at irregular intervals and in much smaller numbers. The serum agglutination titre of the immunised goat was 1 : 512 at the time of inoculation of the test dose and did not increase, and the whey agglutination titre was fairly steady for both sides of the udder at about 1 : 32. In the control animal agglutinins at 1 : 2 were detected in the whey of the infected side only, for a few days in the second week; the serum titre rose from nought at the time of infection to 1 : 64 two weeks later.

In contrasting Figures 4 and 5 we have dealt with the individual daily measurements, but we have admitted that Fig. 5 represented a marked reactor and not an intermediate or minimal one. To consider the full results it was necessary to take into account all the immunised and all the control goats. This involved consideration of a very large number of figures. In view of this, three sets of measurements were chosen to establish the protection given by vaccination, namely those during the first and second weeks after inoculation of the test dose for milk yield, neutrophil count in milk, and concentration of S.13 in milk. The figures were dealt with as explained under " Materials and Methods ", and are recorded in Table II. Measurements for the third week, and for later periods, showed that protection due to vaccination was somewhat less obvious owing to the partial recovery of some of the control goats. Comparisons of the control and vaccinated groups for the second week after infection are presented in Fig. 6, which shows very clearly the protection afforded by vaccination.

Although it was decided to consider all the vaccinated groups together, it should be pointed out that all the animals in group 8 (Table II) became lame following intravenous inoculation, and that this method of immunisation therefore appears impracticable. In group I there may well have been some association between the poorer protection afforded by vaccination and the short interval that was left between vaccination and inoculation of the test dose.

Antibody titre. The agglutination titre of the blood serum at the time of exposure of immunised animals to the test dose was commonly 1 : 512 or 1 : 1,024, and the titre of the milk whey 1 : 16. In some animals there was a small rise in the agglutination titre of serum and whey during the first fortnight after inoculation of the test dose; maximum values recorded were 1 : 8,192 for serum and 1 : 128 for whey.

There was no obvious correlation between the blood serum antibody titre as judged by the mouse test or the agglutination test

at the time of exposure of immunised animals to the test dose, and the degree of resistance shown by these animals as judged by the milk yield of the inoculated side of the udder. The only slight correlation noted was the following: —

Milk yield	*Mouse deaths out of ten*
100% or over	... 0 or 1
80% to 99%	... 0 to 6
below 80%	... 3 to 8

Blood leucocytes. Multiple samples were taken on the day of infection, after which four goats were sampled twice a day and another four once a day. All the goats showed a fall in leucocytes and among those sampled twice a day a leucopenia was observed at 30, 54, or 96 hours after infection. There was no association between observed leucopenia and protection against the test dose.

Protection given by previous infection with S.13 in one side of the udder

In order to test whether resistance to streptococcal infection at a later date would develop as a result of an experimentally produced attack of streptococcal mastitis, seven goats were inoculated into one side of the udder with S.13 and the disease was allowed to progress untreated. In two of these animals the streptococcus had persisted in the udder for four months, but was not isolated from any of more than twenty samples of milk taken over the five succeeding months. In the other five animals the organism persisted through the dry period and into the succeeding lactation.

Between six and eight months after the first intramammary inoculation all these seven animals were exposed to a second test dose of the same strain, this time given into the non-infected side of the udder. The figures for milk yield, milk neutrophil counts, and streptococcal counts for this group of animals are given in Table II, and average measurements are shown diagrammatically in Fig. 6. There appeared to be some degree of protection against the second test dose, but it did not appear to be as high as in vaccinated animals.

Exposure of animals, previously inoculated with serum obtained from immunised goats, to an intramammary test dose

To test the possibility of producing passive immunity against an intramammary test dose of strain S.13, three animals were inoculated subcutaneously with 100 ml. immune serum and were exposed to a test dose 18 hours later. The immune serum was obtained from nine animals actively immunised against strain

Table II

Results of Milk Examination on Protected and Unprotected Goats, as Daily Averages for the First and Second Weeks Following an Intramammary Test Dose Of S.13.

		Inoculations at weekly intervals	*Interval between end of immunisation and infection*	*YIELD per cent. normal*		*NEUTROPHIL COUNT millions per ml.*		*S. 13. COUNT thousands per ml.*	
				Week 1	*Week 2*	*Week 1*	*Week 2*	*Week 1*	*Week 2*
RESISTANCE ACQUIRED BY VACCINATION	1	4 of 10 ml. i.p.	1 Week	84	88	0·1	0·4	0·3	0·6
				62	67	4·8	2·1	0·1	0·1
				70	42	0·6	3·2	1·7	0·0
				86	73	0·0	0·1	3·0	13·6
	2	4 of 10 ml. i.p.	8 Weeks	100	95	0·0	0·0	0·0	0·1
				93	88	0·0	0·0	0·1	0·0
	3	4 of 10 ml. i.p. then 4 of 10 ml. sc. + 0·4 ml. i.d.	1 Week	94	98	0·1	0·0	0·9	1·3
				98	94	0·0	0·0	0·0	0·7
				73	97	1·1	1·2	7·0	30·1
				100	97	0·0	0·0	0·0	0·9
	4	Same but 4 week interval between	1 Week	90	98	0·0	0·0	0·0	0·0
				78	91	0·2	0·0	0·0	0·0
				87	94	0·2	0·6	0·0	0·0
	5	4 of 10 ml. i.p. 4 weeks then 4 of 10 ml. sc.	1 Week	88	94	0·1	0·1	0·0	0·0
				94	92	0·1	0·0	0·0	0·0
				94	92	0·1	0·0	0·0	0·0
	6	4 of 10 ml. i.p. 4 weeks then 4 of 0·4 ml. i.d.	1 Week	97	102	0·0	0·0	0·0	0·0
				93	95	0·0	0·0	1·0	0·0
				93	95	0·0	0·0	0·2	0·0
	7	4 of 10 ml. i.p. 4 weeks then 4 of 10 ml. i.p.	1 Week	97	101	0·0	0·0	0·0	0·0
				82	88	0·0	0·1	0·0	0·0
				83	82	0·0	0·0	0·0	0·0
	8	4 of 0·5 ml. i.v. + i.p. + i.d.	2 Weeks	83	94			16·9	3·4
				101	85			0·0	0·0
				84	86			0·0	0·0
				106	119			0·0	0·0
			Averages	89	90	0·3	0·1	1·2	1·9
RESISTANCE ACQUIRED BY INFECTION		Infected in other side of udder 6 to 8 months before test dose		75	50	4·2	6·1	8·1	14·6
				70	76	3·8	1·3	8·9	15·1
				72	83	1·8	2·5	8·9	30·1
				52	52	1·4	2·4	14·9	32·44
				62	73	4·5	1·5	64·3	24·3
				81	73	0·3	0·3	22·6	7·9
				71	76	1·9	0·6	13·1	3·4
			Averages	69	69	2·6	2·1	20·1	18·3

	YIELD per cent. normal		*NEUTROPHIL COUNT millions per ml.*		*S. 13. COUNT thousands per ml.*	
	Week 1	*Week 2*	*Week 1*	*Week 2*	*Week 1*	*Week 2*
INOCULATED WITH 100 ml. OF SERUM FROM RESISTANT GOATS	26	22	30·6	7·8	22·9	22·3
	41	47	21·6	7·4	33·3	9·0
	51	47	11·5	9·8	3·3	9·6
Averages	39	39	21·2	8·3	19·5	13·6
CONTROLS. (NO DETECTABLE ANTIBODY IN BLOOD BEFORE TEST DOSE)	62	87	15·1	7·3	56·4	33·6
	43	42	26·5	29·0	70·0	99·9
	21	7	19·5	17·8	89·3	17·4
	83	51	5·7	15·4	48·1	99·9
	43	10	5·0	32·9	73·9	78·1
	78	89	3·1	1·7	31·7	24·4
	28	38	8·9	10·8	43·3	34·4
	51	12	10·6	23·1	77·9	47·3
	72	75	3·3	0·7	22·7	30·1
	9	2	35·0	27·4	51·0	12·6
	22	8	3·8	24·9	7·6	18·6
	70	44	8·9	20·6	71·1	6·1
	25	2	27·5	25·2	74·9	8·3
	46	51			76·9	48·7
	27	5			77·1	82·1
	54	24			82·1	82·1
	81	93			37·4	37·4
	64	15				
	68	88				
	78	59				
	46	33				
	8	6				
	29	8				
	92	57				
	60	19				
	57	15				
	41	36				
	41	24				
	75	69				
	86	76				
	46	16				
	60	42				
	15	7				
	49	31				
	25	9				
	97	106				
Averages	51	38	13·3	18·2	58·3	44·7

S.13; the pooled serum from these animals had an agglutination titre of 1 : 512 and was not filtered before inoculation.

Eighteen hours after subcutaneous inoculation of the serum, i.e., at the time that the intramammary test dose was given, the three injected animals showed a blood serum titre of 1 : 4, but the milk whey was negative in each case. The blood titre remained at 1 : 4 and the whey was negative over the three-week period of observation after infection.

The results for this small group of animals are given in Table II, and average measurements for the second week after inoculation are shown in Fig. 6. Taken as a group these animals could not be distinguished from controls, but in one goat low streptococcal counts below those recorded for any of the controls in the first week suggested a possible effect of inoculation with immune serum.

FIG. 6

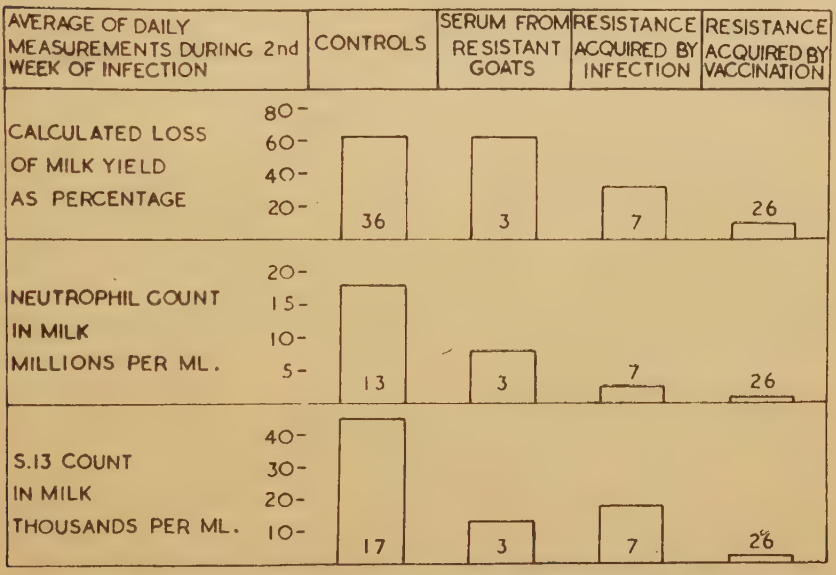

FIGURE 6. Average figures for all vaccinated and control animals for milk yield, milk neutrophil counts, and streptococcal counts for the second week after exposure to an intramammary test dose of S.13. The figures in the columns refer to the number of animals on which measurements were made.

Observations on a Relationship between Milk Yield, Body Temperature, Streptococcal Count in Milk, Neutrophil Count in Milk and Leucocyte Count in Blood

During the course of these experiments it became obvious that there was more than a casual relationship between a sudden fall in milk yield and sudden rises in streptococcal count per ml. of milk, neutrophil count per ml. of milk and in body temperature;

these readings coincided with each other in such an overwhelmingly large number of instances that an important correlation could not be doubted. Further, examination of all the available data showed that the rise in streptococcal count preceded by a few hours the rises in temperature and neutrophil count in milk and the fall in milk yield.

Holman, Pattison and Taylor (1950) have given a description of the relationship of abnormally high body temperature and high streptococcal count in milk, to an increase in the lethal power for mice of strain S.13 isolated from the milk at this time. Pattison and Holman (1951) have also noted that streptococci, in relatively small numbers, could sometimes be isolated from the supramammary lymphatic gland of animals destroyed during a period of raised body temperatures. At such a period of crisis in the disease streptococci were penetrating from the duct system, through the walls of the acini, into the lymphatic channels (Pattison, 1951). The opinion was expressed that the period of penetration was brief, being measured usually in hours.

If all this evidence is considered together, a logical conclusion appears to be that from time to time in the course of this form of mastitis, under some stimulus as yet undetermined, the streptococcus begins to multiply rapidly and to produce variants of increased virulence. In spite of the release from blood to milk of great numbers of neutrophils (Taylor, *et al.*, 1950), the defences of the udder are overwhelmed and the organisms penetrate to the lymphatic system, where they are destroyed with great rapidity. This period of local strife can be measured as a sharp fall in milk yield and is reflected in the body as a whole by a rise in temperature. This process is part of the characteristic pattern of the disease.

Sudden multiplications of streptococci, associated with related rises in body temperature and in neutrophil count in the milk, and with falls in milk yield, were noted from time to time in immunised animals. The degree of immunity shown by any individual appeared to be a measure of the success with which the damaging effects of this streptococcal multiplication were overcome. In successfully immunised animals the fall in milk yield and the rise in temperature were slight, but the rise in neutrophil count, though transitory, might be quite marked. It is concluded, therefore, that sudden multiplication of streptococci within the duct system can occur in non-immune and immune animals, but that the immune animal escapes the udder damage that is likely to occur in the non-immune animal. The reason for the sudden multiplication of streptococci within the duct system remains unknown, but there is little doubt that it initiates the sequence of events detailed above. Elucidation of this problem would be a valuable contribution towards complete understanding of the disease.

DISCUSSION

The literature indicates that there is no entirely acceptable experimental evidence to show that any form of mastitis in any species can be prevented, or controlled, by the use of vaccines. Bryan (1946) has pointed out that the majority of observations on vaccination in bovine mastitis were made prior to 1936; these have been summarised by Munch-Petersen (1938).

In view of this considerable volume of equivocal evidence on the efficacy of vaccination in the control of mastitis in the field, the experiments reported in this paper were designed to examine only the vaccination routine most likely to be successful, namely inoculation with multiple doses of living culture of one strain of streptococci and subsequent exposure to a test dose of the same strain.

The considerable measure of success obtained in these experiments raises the question of why so many previous investigators have reported unfavourably on the use of vaccines in the control of bovine mastitis. Three reasons appear important. Firstly, no investigator appears to have measured detectable antibodies as a guide to the immunisation of the animal. Secondly, with few exceptions, experiments depended for their result on exposure of vaccinated animals to haphazard infection under field conditions, the assumption being that one strain of *Str. agalactiae* would protect against all other strains. From work in mice it seems likely that streptococcal immunity is, to some extent at least, type-specific within group-B (Pattison, 1948), and at present it cannot be assumed that vaccination with any one strain will give protection against all others. Thirdly, the very nature of chronic streptococcal mastitis and the great importance of individual susceptibility to this disease, make the interpretation of vaccination experiments difficult unless large numbers of animals are used; this point has already been discussed in this paper and attention has been drawn to the erroneous conclusions that can easily arise from experiments involving only small numbers of animals.

The finding that an attack of streptococcal mastitis in one side of the goat's udder produced only a moderate degree of protection against subsequent infection of the other side with the same strain of organism, is interesting in the light of studies made by Hughes (1945) on immunological reactions in localised diseases in man. He found that a variety of localised human infections caused little or no immune response in the body as a whole, and concluded that "measures designed to enhance the resistance of the body" were indicated in conditions of this type.

The experiments reported here showed that a high degree of protection against an intramammary test dose of *Str. agalactiae* strain S.13 could be conferred on goats by subjecting them previously to a series of parenteral inoculations of living culture of the

same strain. These results may well be applicable to the cow. McEwen and Cooper (1947) have reviewed some of the problems connected with the immunisation of cows against mastitis and have emphasised their complexity. These studies in goats support many of their views. But in spite of the obvious difficulties still to be overcome we cannot agree with Francis (1947) that further experiments on the possibility of controlling bovine mastitis by vaccination are not worth while. It appears to us that this field of enquiry might well yield results of practical importance.

SUMMARY

A passive protection test in mice and a slide agglutination test were used to follow the production of streptococcal antibody in goats inoculated with *Str. agalactiae* strain S.13.

Antibodies were formed during the course of the disease induced by inoculating living streptococci into one side of the udder, but the time of their appearance and the amount produced varied from goat to goat. When the blood serum agglutination titre was as high as 1 : 8, agglutinins could usually be detected in the milk whey from both sides of the udder.

Four intraperitoneal inoculations of various doses of living broth culture caused production of streptococcal antibody. The amount of antibody produced and its persistence appeared to be related to the amount of culture inoculated. Four inoculations of 10 ml. produced a fairly high level of antibody, detectable up to at least eight months after inoculation.

In the case of two goats in which a good level of antibody was produced by a series of intraperitoneal or intravenous inoculations of living culture, the antibody level remained approximately the some over a period of two and a half years.

Vaccination of twenty-six animals by various routes with a series of inoculations of living culture of *Str. agalactiae* strain S.13 produced a high level of resistance to an intramammary test dose of the same strain, when compared with thirty-six control animals. Resistance to the test dose was measured by milk yield, neutrophil counts in milk and counts of streptococci in milk.

In a group of seven animals there was some evidence of resistance to an intramammary test dose given six to eight months after an infecting intramammary dose inoculated into the other side of the udder.

To test passive immunity three animals were inoculated subcutaneously with 100 ml. of serum from immunised goats and were exposed to an intramammary test dose eighteen hours later. Their reaction could not be distinguished from that of control animals.

A close relationship was noted between sudden rises in streptococcal count in milk, rises in body temperature and in neutrophil count in milk, and falls in milk yield and in the blood

leucocyte count. The opinion is expressed that these findings indicated a period of crisis in the disease and that each crisis began as a sudden multiplication of streptococci within the udder.

Great importance is attached to individual idiosyncrasy in the reaction of goats to an intramammary inoculation of living streptococci. It is considered essential to carry out vaccination experiments on a sufficiently large number of animals to allow for these wide variations in susceptibility.

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CHEMOTHERAPEUTIC RESPONSE OF THE ORGANISM CAUSING ENZOOTIC ABORTION IN EWES

By

J. Francis*

Imperial Chemical Industries Limited, Biological Laboratories, Wilmslow, Cheshire.

INTRODUCTION

Enzootic abortion of ewes was described by Stamp, McEwen, Watt and Nisbet (1950). The foetal membranes resembled those of the bovine infected with *Brucella abortus,* but the uterine mucosa and cotyledons did not show gross changes. Smears made from the foetal cotyledons, chorion or exudate revealed large numbers of small, red organisms when stained with a modified Ziehl-Neelsen technique. These minute organisms, which were easily overlooked unless in clusters, also stained by the Macchiavello and Castaneda methods for rickettsiae, and by Giemsa. It was stated that the morphology and staining reactions of the organism resembled those of the rickettsiae and the psittacosis-lymphogranuloma group of infective agents. It grew readily when inoculated into the yolk-sac of six- to seven-day-old embryos, killing them within four to nine days. Mice inoculated intranasally with heavily infected suspensions of yolk-sac died five to seven days later. Their lungs showed areas of pneumonia containing numerous organisms. The infection was transmitted in series from mouse to mouse, but no obvious disease was produced in other laboratory animals. One cow, in the sixth month of pregnancy, inoculated intravenously with a suspension of infected foetal membranes from a sheep, aborted ten weeks after inoculation. The macroscopic lesions were indistinguishable from those caused by an infection with *Br. abortus*, and smears prepared from the foetal membranes showed elementary bodies singly and in clusters but less abundant than in the necrotic foetal membranes from sheep.

There is now good evidence that the organism is a member of the psittacosis-lymphogranuloma group (Stamp, 1951; Barwell and Bishop, 1951). When the present work was performed, however, the classification was uncertain, and it was felt that if the chemotherapeutic response to the organism resembled that of the rickettsiae it would be valuable in the search for compounds active against this latter group. It will be shown, however, that the chemotherapeutic response resembled that of the psittacosis-lymphogranuloma group, a fact which supports the other evidence on classification.

* Present address : Department of Preventive Medicine, University of Queensland Veterinary School, Brisbane.

EXPERIMENTAL

Infected yolk-sacs, received from Dr. A. D. McEwen, were passed in seven-day-old embryos, which died, on the average, five days after infection. A series of experiments was carried out in which various therapeutic agents were injected into the yolk-sac about 20 minutes after the organism. Embryos were examined daily and smears made from the yolk-sacs of those that died; observations were continued for 11 days. The results in different experiments, summarised in Table I, were very similar. It will be seen that sulphone (4:4′diaminodiphenyl sulphone), *p*-aminobenzoic acid and streptomycin were inactive. Large doses of sulphathiazole and penicillin had slight activity. Chloromycetin was active in doses of 0·5 to 3 mg. The survival time of embryos receiving 9 mg. was less than those receiving 3 mg. because of deaths due to toxicity. Aureomycin was active at doses of 0·05 to 9 mg., the best result being obtained with 1 mg. Terramycin was active at doses from 0·00625 to 2 mg., the best result again being obtained with a dose of 1 mg.

Results when aureomycin was given intra-allantoically

In nearly all work on the chemotherapy of the rickettsiae and the psittacosis group of organisms drugs have been injected into the yolk-sac; this technique has been followed in the main part of the present work. It has been pointed out, however (Francis, 1946) that, when organisms are inoculated on to the chorio-allantoic membrane, it is a much more exacting test of a compound than if it is injected into the yolk-sac instead of on to the membrane. Thus, when streptococci were inoculated on to the chorio-allantois, antiseptic substances such as proflavine were active only when they also were inoculated on to the chorio-allantois, but the sulphonamides and penicillin, which have a systemic effect, were active when injected into the yolk-sac, as well as when applied locally.

When the position is reversed and organisms are injected into the yolk-sac, as in the present work, it would seem that injection into the allantoic cavity would provide a more severe test of the compound. In one experiment a series of eggs were given aureomycin by this route and the following average survival times, in days, were obtained in groups of five eggs: —

Dose of aureomycin						
0.5 mg.		*1 mg.*		*2 mg.*		*Controls*
A	*YS*	*A*	*YS*	*A*	*YS*	
11·4	14·0	10·4	13.2	10·6	11·8	4·2

A = aureomycin injected into the allantoic cavity.
YS = aureomycin injected into the yolk-sac.

TABLE I

AVERAGE SURVIVAL TIME IN DAYS OF CHICK-EMBRYOS INOCULATED INTO THE YOLK-SAC WITH INFECTIVE MATERIAL FOLLOWED 20 MINUTES LATER BY VARIOUS THERAPEUTIC AGENTS[1].

Sulphone								*Streptomycin*							
0·33 mg.		0·5 mg.		1 mg.		2 mg.		1 mg.		3 mg.		6 mg.		10 mg.	
No.[2]	S.T.	No.	S.T.	No.	S.T.	No.	S.T.	No.	S.T.	No.	S.T.	No.	S.T.	No.	S.T.
4	4·75	9	5·5	16	5·0	5	3·0	8	4·25	4	4·5	14	5·5	10	5·7

Sulphathiazole				*P-aminobenzoic acid*				*Penicillin*							
5 mg.		10 mg.		10 mg.		20 mg.		200 u.		500 u.		1,000 u.		2,000 u.	
5	4·4	15	6·7	9	4·22	8	4·12	8	4.12	9	5·77	13	6·6	15	7·7

Chloromycetin															
0·02 mg.		0·1 mg.		0·2 mg.		0·5 mg.		1 mg.		2 mg.		3 mg.		9 mg.	
3	5·66	14	5·2	5	5·8	19	7·3	9	8·3	11	10·1	12	7·58	4	5·75

Aureomycin																	
0·02 mg.		0·05 mg.		0·1 mg.		0·2 mg.		0·5 mg.		1 mg.		2 mg.		3 mg.		9 mg.	
9	5·4	10	9·6	12	7·6	5	11·0	14	11·8	5	13·2	5	11·8	8	9·12	8	7·62

Terramycin												*Controls*	
0·00625 mg.		0.0125 mg.		0·025 mg.		0·1 mg.		0·5 mg.		1 mg.		2 mg.			
11	7·5	4	8·5	15	11·0	9	10·4	8	13·1	5	14·0	5	10·0	35	5·0

1. In calculating the survival time eggs that were alive 11 days after infection were allotted a survival time of 14 days.
2. No. = number of eggs inoculated.
   S.T. = average survival time.

Aureomycin is a drug with systemic activity and as would be expected it was effective when given into the allantoic cavity. The same might not be true of all compounds.

DISCUSSION

Greiff, Pinkerton and Moragues (1944) and Hamilton (1945) found that 2 to 6 mg. of *p*-aminobenzoic acid were active against typhus rickettsiae in the chick-embryo, whereas Hamilton found that it had no action on lymphogranuloma or psittacosis organisms. It will be seen that in its reaction to *p*-aminobenzoic acid the ewe-abortion organism behaves like the other members of the psittacosis-lymphogranuloma group of organisms and not like rickettsiae. The same is true of its response to streptomycin, which is active against rickettsial infections in eggs, mice, and guinea-pigs (Smadel, Jackson and Gauld, 1947) but has no effect on the psittacosis-lymphogranuloma group (Hamre and Rake, 1947).

The sulphonamides have no action against, and may even stimulate, the rickettsial infections, but according to American authors they have a definite action against lymphogranuloma venereum, and a slight action on psittacosis (see MacCallum, 1950). Hurst, Peters and Melvin (1950) found that "Sulphamezathine" and sulphadiazine had a slight action on lymphogranuloma venereum in the chick-embryo in some experiments but not in others. Sulphathiazole is rather more active than the two foregoing sulphonamides at equal concentrations, and there may be some specificity of action between the different sulphonamides (Francis, 1951) which could account for the definite effect with sulphathiazole observed in the present series of experiments. Sulphone, which was included because Summers (1949) had shown it had a high activity against toxoplasmosis in mice, proved to be inactive.

The reaction of the various organisms to the foregoing therapeutic agents can be classified as follows: —

	Rickettsiae	*Ewe abortion organism*	*Psittacosis-lymphogranuloma*
p-aminobenzoic acid ...	+	−	−
Sulphonamides	−	+	±
Streptomycin	+	−	−

A study of the chemotherapeutic response therefore led to the same conclusion on the classification of the ewe-abortion organism as that reached by the standard biological methods (Stamp, 1951; Barwell and Bishop, 1951).

Penicillin (Grieff and Pinkerton, 1944) and the newer antibiotics (MacCallum, 1950) are active against all the above organisms, so the results are of little value for the purpose of classification. Hurst, Peters and Melvin (1950) found that both terramycin and aureomycin were highly active against lymphogranuloma venereum in the chick-embryo, the former more so; chloramphenicol took third place and penicillin (in water) fourth. It will be seen that this order is the same as that obtained in the present study against the ewe-abortion organism. Aureomycin acts on an earlier stage of the life cycle of the feline pneumonitis organism than penicillin (Gogolak and Weiss, 1950), and this may partly account for its greater activity against this group of organisms.

Despite the high activity of the newer antibiotics against the ewe-abortion organism it seems unlikely that chemotherapy will play a major part in the control of the disease. McEwen, Stamp and Littlejohn (1951) showed that an active immunity could be produced by formolized vaccine made from infected yolk-sacs or ovine foetal membranes and this would seem to to be a much more practical method of control.

SUMMARY

The reaction of the ewe-abortion organism to various therapeutic agents was tested in the chick-embryo. It was slightly susceptible to the action of sulphathiazole and penicillin, but resistant to the action of streptomycin, *p*-aminobenzoic acid and sulphone (4 : 4′ diaminodiphenyl sulphone). Certain of these reactions support the view that the organism belongs to the psittacosis-lymphogranuloma group.

Chloromycetin, aureomycin and terramycin were more active than any of the foregoing agents.

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A STUDY OF THE *IN VITRO* AND *IN VIVO* ACTION OF CERTAIN ANTIBIOTICS

By

S. J. Edwards

Agricultural Research Council, Field Station, Compton, Berks.

INTRODUCTION

In the recent literature a number of reports have appeared dealing with the sensitivity of organisms to individual antibiotics but so far little work has been published showing a comparison of their sensitivity to several substances under the same conditions of testing. It is generally accepted that such *in vitro* tests should precede the rational selection of antibiotics for chemotherapeutic use and that they have the advantage of being simple to perform and of yielding quick results. Up to the present, however, there has been little confirmation of the value of *in vitro* results by *in vivo* tests in a susceptible laboratory animal so that it cannot be said that adequate confirmatory tests have always been made before applying individual antibiotics to specific clinical infections.

The work described in the present paper was designed to demonstrate whether there is any correlation between *in vitro* sensitivity and therapeutic activity *in vivo* and for this certain organisms were selected for detailed study in experimental infections in mice. The organisms examined belonged mainly to those species responsible for bovine mastitis since the main object of the work was to examine their sensitivity to certain antibiotics with a view to the further development of the therapy of this disease.

METHODS

Antibiotics tested. The antibiotics studied were penicillin, aureomycin, streptomycin and nisin while chloromycetin was tested on a limited scale. Throughout the *in vitro* work, the standard calcium salt of penicillin containing 1059 units per mg. was used; in the *in vivo* work the sodium salt of penicillin G having a potency of 1650 units per mg. was used. Aureomycin was employed as the crystalline hydrochloride containing 970 microgramme of the pure salt per g. and streptomycin as the pure dihydrostreptomycin hydrochloride. A sample of crystalline nisin containing 30×10^6 units per g. was obtained from Dr. Hirsch, N.I.R.D., Shinfield. In view of the different values attributed to the individual unit in the case of penicillin and nisin, solutions of the antibiotics for *in vitro* and *in vivo* work were always made to contain known weights expressed as micrograms per millilitre (μg./ml.) of each substance. All solutions were made in sterile distilled water except in the case of nisin which was dissolved in N/50 HCl.

IN VITRO ACTION

Bactericidal effect in milk

Methods. The technique used for testing the sensitivity of organisms was similar to that used previously (Edwards, 1949) in the case of *Str. agalactiae*. It was found that strains of this organism gave a uniform bactericidal end point after contact in sterilised separated milk for 24 hours at 37° C. in the presence of penicillin. It was thought therefore that this would prove a satisfactory method for comparing the inhibitory action of other antibiotics on different organisms. Each organism to be tested was grown for 18 to 24 hours at 37° C. in Hartley broth and appropriate dilution made in sterile saline as follows:—(*a*) for streptococci 1/500 (*b*) for staphylococci, coliform and salmonella strains 1/10,000. From these dilutions 0.1 ml. was added to 9.9 ml. of separated milk sterilised by three successive steamings. The number of organisms contained in 1 ml. of milk varied from 4,000 to 10,000 as tested by a preliminary count. The final concentration of antibiotic in the sterile milk ranged from 0.02 to 200 μg./ml.

After incubation for 24 hours at 37° C., one drop (0.02 ml.) was removed from milks containing antibiotic dilutions and also from a control milk and inoculated on to a blood-agar plate in which colony counts were made. The number of strains of each organism tested against each antibiotic was usually five except in the case of certain organisms, viz.:—*Str. agalactiae* (26 strains), *Str. uberis* (17 strains) and *Staph. aureus* (32 strains).

Results.

It will be seen in Table I that penicillin surpassed any of the other antibiotics against Gram-positive organisms. Against *Str. agalactiae* and other mastitis streptococci and staphylococci, all strains tested showed a uniform sensitivity ranging from 0·02 μg./ml. to 0·08 μg./ml. This uniformity in sensitivity agrees with the findings of previous workers who have used the same technique (Brownlee 1945, Watts and McLeod, 1946) and confirms the view (Edwards, 1949) that the action of penicillin is bactericidal rather than bacteriostatic on sensitive organisms growing rapidly in a favourable medium. The action of nisin on *Str. agalactiae* and *Str. uberis* was also bactericidal at moderately low concentrations, thus confirming the original work of Mattick and Hirsch (1947) in respect of the sensitivity of these organisms; other streptococci proved less sensitive to nisin. The performance of aureomycin and streptomycin in this test was generally poor against all organisms while penicillin and nisin, as might be anticipated, were effective only in high concentration against the Gram-negative organisms. In contrast to the sharp bactericidal end-points and narrow range shown by penicillin against all strains of the Gram-positive organisms, the other antibiotics generally did not produce clear-cut results and their effective end-points varied widely with different strains. The action of aureomycin seemed to be bacteriostatic rather than bactericidal against

most organisms, thus confirming the findings of Chandler and Bliss (1948) and Price, Randall and Welch (1948), who also found that aureomycin brought about a progressive decline in the bacterial population for the first 24 days of exposure but that it increased thereafter.

Bacteriostatic action in broth and in milk

In the previous test it was clear that the inhibitory concentrations of the antibiotics, excepting penicillin, were much higher than those obtained by other workers. The tests employed by them, however, have generally denoted only the bacteriostatic effect of the antibiotics in various media and cultures were not made to determine the viability of the test organism after contact. In only one instance does there appear to have been an attempt to investigate the bactericidal activity of an antibiotic, viz. in the experiments described by Price *et al.* (1948) in the case of aureomycin. They found that the concentration at which no viable cells remained was markedly higher than that which prevented growth.

In order that the present enquiry should be more strictly comparable with that of other workers, the inhibitory concentrations of the antibiotics were determined in broth, using Hartley broth, and the end-point regarded as that preventing visible turbidity after 24 hours contact with varying concentrations at 37° C. Comparison with the end-point obtained in milk with brom-cresol-purple as indicator was also made. A standard inoculum of the organism corresponding to that used by May, Voureka and Fleming (1947) was used.

Methods. Tests were carried out by a serial dilution method similar to that employed by May, *et al.* (1947), Price, *et al.* (1948) and by Hughes and Farmer (1950). Organisms were tested against varying ranges of antibiotic concentration according to the sensitivity shown by each species in a preliminary test. A stock solution of antibiotic having a concentration of 2,000 μg./ml. was prepared and from this a further primary dilution was made in sterile broth and in milk to give a concentration suitable for setting up progressive twofold dilutions in a series of 3 in. by ½ in. sterile tubes. Into each of these tubes was placed 1 ml. of sterile broth or milk and then 1 ml. of the antibiotic dilution into the first tube and falling twofold dilutions made in the remainder of the series. The organism under test was grown for 18 hours in Hartley broth and its opacity adjusted to that of tube 1 in Brown's scale. The culture was diluted with four times its volume of broth and one drop (.02 ml.) added to each serial dilution. The final dilution of culture in each tube was therefore 1 : 200. Control tubes on growth of the organisms and sterility of the antibiotic were also included in each series. Five strains of each species of organism were tested against each antibiotic.

Results.

In broth and in milk, penicillin was the most active of the four

Table I

Bactericidal Action of Antibiotics in Sterile Milk.

Concentrations (ug/ml.) lethal to various organisms after contact for 24 hours at 37°C.

Antibiotic	*Str. agalactiae*	*Str. uberis*	*Str. dysgalactiae*	*Str. zooepidemicus*	*Staph. aureus*	*Bact. coli.*	*Sal. dublin.*
Penicillin...	0·04 0·08	0·04–0·06	0·02	0·04 0·08	0·04–0·08	40–100	20–40
Aureomycin	>40	60–>60	5–100	5–40	40–>60	40–100	150
Streptomycin	200	>200	40–80	60–100	20–100	100	>200
Nisin	0·5–10·0	0·5	40–>40	5–20	10–80	>200	>200

TABLE II

BACTERIOSTATIC ACTION OF ANTIBIOTICS IN HARTLEY BROTH AND IN MILK.

Concentrations (ug./ml.) inhibitory to various organisms after contact for 24 hours at 37° C.

Antibiotic	*Medium*	*Str. agal-actiae*	*Str. dysgal-actiae*	*Str. uberis*	*Str. zoo-epidemicus*	*Staph. aureus*	*C. pyogenes*	*Bact. coli.*	*Salm. dublin.*
Penicillin	Broth	0·01 0·04	0·0025–0·005	0·01–0·03	0·008–0·02	0·02 –0·06	0·003–0·05	25 100	6·0
	Milk	0·02 0·04	0·01 –0·02	0·03–0·06	0·001–0·008	0·004–0·01	0·003 0·006	50 100	3·0
Aureomycin	Broth	0·02–0·16	0·04–0·3	0·02–0·3	0·04–0·2	0·05–0·6	0·05 25	6·0–12·0	3–12·0
	Milk	0·08–0·16	0·3 –0·6	0·3 –0·6	0·08–0·2	0·08–0·2	0·2 –6·0	3 - 12·0	6·0
Streptomycin	Broth	1·0–20	1·0 10	1·0 20	1·0–5	2·0–10	0·1–3·0	6·0–12·0	200
	Milk	100 200	50–100	200	25–100	6·0–100	0·4 12·0	25–50	200
Nisin	Broth	0·01–0·3	0·6 2·0	0·01 0·08	0·008–0·06	2·0–10	0·2–3	[>200	>200
	Milk	6 12	50 100	0·1–0·4	0·6 40	6·0–100	1·0 6·0	>200	>200

antibiotics against streptococci and staphylococci, the bacteriostatic end points being almost identical with the lethal end points shown in Table I. The activity of aureomycin against these organisms was only slightly less than that of penicillin but they were comparatively resistant to streptomycin. The resistance of Gram-positive organisms to streptomycin has also been demonstrated by Robinson (1946), Coles (1948) and by Hughes and Farmer (1950). The inhibitory action of nisin was similar to that described by Mattick and Hirsch (1947) and appeared to be confined to the streptococci though its action was less effective in the presence of milk. Strains of *Bact. coli* were sensitive only to streptomycin and aureomycin while *Salm. dublin* was inhibited by penicillin and aureomycin. (Table II.)

It is difficult to compare the *in vitro* results of the present study with those of other workers because of the different methods employed, although they agree closely with those of Long, Bliss, Schoenbach, Chandler and Bryer (1950). Hughes and Farmer (1950) have drawn attention to the fact that different values are obtained according to the technique employed, while the influence of medium on the end-point has been demonstrated by May *et al.* (1947) who found that a greater concentration of streptomycin was required to inhibit organisms when tested in a broth medium than in a serum-water medium or in diluted broth. In the present experiment it will be seen (Table II) that while the action of streptomycin and nisin was greatly reduced in the presence of milk, penicillin and aureomycin seemed to retain most of their efficiency.

IN VIVO THERAPEUTIC ACTION IN MICE

In order to compare the findings of the *in vitro* sensitivity of organisms to various antibiotics with their suceptibility *in vivo,* a study was made of their therapeutic effect on certain experimental infections in mice. It must be stressed that the study was not intended to investigate the full therapeutic potential of any of the antibiotics but to compare their protective effect only under standard conditions.

Methods. A relatively simple technique was used in the first place similar to that devised by Bryer, Schoenbach, Bliss and Chandler (1948) for the comparison of the efficiency of penicillin and aureomycin in experimental infections in mice. Immediately following the intraperitoneal injection of culture of the organism under test into white mice a standard dose of 50 mg./kg., i.e. of 1 mg. for mice of 20 grams weight, was introduced subcutaneously. The survival rate of mice was observed over a period of seven days and the heart blood of all mice dying during this period and of all survivors was cultured for the presence of infection. While this simple technique served to show the salient difference in protective value of the antibiotics against certain infections it was found that, with others, it was necessary to vary the technique in such a manner as to include larger or multiple dosage of the antibiotics.

It was not possible to investigate nisin fully since the supply of pure substance was limited and a sample of impure substance containing 5×10^6 units per g. killed 50 per cent. of mice using a 50 mg./kg. dose : chloromycetin was only tried in salmonella infection.

Solutions of antibiotics were usually prepared in a standard strength of 2 mg. per ml. and the potency of different solutions checked before use against organisms of known sensitivity.

Streptococcal Infection

Str. zooepidemicus (Lancefield Group C). A strain, originally isolated from the secretion of a cow affected with mastitis, was chosen. This strain (Strep. 140) was virulent for mice, all mice dying within 24 hours when 0·25 ml. of an 18 hours Hartley broth culture was injected intraperitoneally. The number of organisms in this culture was found to be about 100×10^6 per ml. The results of a preliminary experiment are shown in Table III.

TABLE III

PROTECTIVE VALUE OF A SINGLE DOSE OF ANTIBIOTICS AGAINST STREP. 140.

Antibiotic	*Dose*	*No. Mice*	*Death rate during seven days* 1 2 3 4 5 6 7	*No. Survivors*
Penicillin Ca. ...	... 1 mg.	10	8 2	0
Penicillin G. ...	...	,,	0 10	0
Aureomycin ...	...	,,	2 0 2 0 2 0 0	4
Nisin	...	,,	10 0	0
Streptomycin ...	...	,,	10 0	0
Penicillin Ca. ...	... 2 mg.	10	4 6	0
Penicillin G. ...	...	,,	0 8 2	0
Aureomycin ...	...	,,	0 1 2 2 0 1 0	4
Nisin	...	,,	6 2 0 0 0 0 0	2
Streptomycin ...	...	,,	6 4	0
Control	... —	20	19 1	0

With the exception of aureomycin, the antibiotics used afforded little or no protection against infection with this streptococcus. It will be noted also that whereas mice died within two days when injected with either penicillin, nisin or streptomycin, the deaths in those treated with aureomycin were delayed and occurred at intervals over six days. There seemed to be no advantage in doubling the standard dose or in using the sodium salt of penicillin G in preference to the calcium salt.

In view of the poor result obtained with a single dose, it was decided to try the effect of repeated dosage in a further attempt to differentiate the protection afforded by the antibiotics. In this respect. Bryer *et al.* (1948) found that small doses of penicillin given three times daily proved more effective against a group A

streptococcus than a single larger dose and Pattison (1949) demonstrated likewise in the case of *Str. agalactiae*. The next experiment, therefore, was designed to test the value of the standard dose of 1 mg. divided into four doses administered at once and at intervals over 24 hours. The results (Table IV) show that the efficiency of all antibiotics was increased by repeated dosage over a period of 24 hours, and confirmed the previous experiment that aureomycin was the most effective antibiotic; penicillin and nisin gave equal results while streptomycin was of little value. It will be seen that penicillin and aureomycin prevented deaths for two days whereas nisin and streptomycin failed to do so.

TABLE IV

PROTECTIVE VALUE OF REPEATED DOSES OF ANTIBIOTICS AGAINST STREP. 140

Antibiotic	*Dose*	*Injection intervals*	*No. Mice*	*Death rate during seven days* 1 2 3 4 5 6 7	*No. Survivors*	*Per Cent.*
Penicillin G. ...	0·25 mg.	0, 8, 16, 24 hours	30	0 0 6 7 2 3 0	12	40
Aureomycin ...			„	0 0 1 2 2 2 0	23	77
Nisin			„	2 11 4 0 0 0 0	13	43
Streptomycin ...			„	4 21 1 0 1 0 0	3	10
Control	—	—	30	5 25	0	0

The relationship between the efficiency of antibiotics and number of streptococci injected.

Further experiments were carried out, using the same technique as that shown in Table IV, with a view to obtaining confirmation of the results. The same dosage of antibiotic was employed, but the protection afforded in succeeding tests varied considerably and at first it seemed difficult to explain the cause of this variation. Not only did the survival rate vary in the mice treated with antibiotics but the death rate in the control mice occurred at different intervals during the first two days after infection.

These experiments were conducted over a long period and for this reason different batches of Hartley broth were used for the preparation of the test inoculum. It was found, moreover, that the different batches of broth yielded growths of streptococci whose viable count varied widely between 15×10^6 and 1400×10^5 organisms per ml. When the results of the several tests performed were arranged according to the number of streptococci in the culture medium, it was found that there was a relationship between the protection afforded by the antibiotic and the number of organisms in the test inoculum.

These results (Table V) show that the efficiency of the antibiotics, especially penicillin, was influenced considerably by the number of organisms in the test inoculum and that as the count increased the protection afforded was reduced.

TABLE V

PROTECTIVE VALUE OF REPEATED DOSES (4 × 0·25 mg.) OF ANTIBIOTICS AGAINST VARYING NUMBERS OF STREP. 140

Batch of Broth	*No. of organisms per ml. culture*	*No. Mice*	*No. Survivors*				*Death rate in controls*	
			Penicillin G.	*Aureomycin*	*Streptomycin*	*Control*	*1st day*	*2nd day*
1	15×10^6	20	9	17	—	0	5	15
2	30×10^6	„	8	15	2	0	2	18
3	100×10^6	„	1	12	—	0	20	0
4	470×10^6	„	0	11	0	0	20	0
5	1400×10^6	„	0	10	0	0	20	0

Staphylococcal Infection

The strain (Staph. 57) used was a coagulase-positive staphylococcus isolated from a subcutaneous abscess on the udder of a cow. An 18-hour culture on an agar slope was suspended in broth and standardised to the opacity of tube 5 in Brown's scale; 0·25 ml. of this suspension injected intraperitoneally into mice proved fatal within 24 hours. The number of organisms in this suspension was about $2{,}500 \times 10^5$ per ml.

A preliminary experiment (Table VI), carried out to find the effect of administering a single dose of antibiotic to mice along with the test inoculum, showed that aureomycin gave good, nisin and streptomycin moderate and penicillin poor protection against staphylococcal infection.

TABLE VI

PROTECTIVE VALUE OF A SINGLE DOSE OF ANTIBIOTICS AGAINST STAPH. 57

Antibiotic	*Dose*	*No. Mice*	*Death rate during seven days* 1 2 3 4 5 6 7	*No. Survivors*
Penicillin Ca.	1 mg.	20	4 7 3 0 0 2 0	4
Penicillin G.		„	5 7 1 1 1 1 0	4
Aureomycin		„		20
Nisin		„	11 0 0 1 0 0 0	8
Streptomycin		„	9 0 0 0 0 2 0	9
Control	—	20	20	0

In view of the fact that the protective action of the antibiotics against streptococcal infection was inversely related to numbers of organisms in the test inoculum, a similar trial was made with Staph. 57.

TABLE VII

PROTECTIVE VALUE OF A SINGLE DOSE (1 mg.) OF ANTIBIOTICS AGAINST VARYING NUMBERS OF STAPH. 57

Density suspension	*No. of viable organisms per ml.*	*No. Mice*	*No. Survivors.*			
			Penicillin G	*Aureomycin*	*Streptomycin*	*Control*
Brown tube 1/2	300×10^6	20	18	20	20	0
2	710×10^6	„	17	20	20	0
5	1550×10^6	„	17	20	12	0
5	2500×10^6	„	4	20	9	0

From Table VII it will be seen that, as with Strep. 140, there is a relationship between mouse protection and the number of organisms injected. Penicillin and streptomycin were least effective against a high number but increased in efficiency with diminishing count although surviving mice at times harboured infection. It is clear that aureomycin was most potent against this infection and its efficiency was not diminished by increasing the bacteria in the test inoculum. In contrast to the study on streptococcal infection where multiple doses were necessary, it was found that a single dose of the antibiotics conferred protection of a sufficiently high order to distinguish their individual potencies against staphylococcal infection.

Bacterium coli *Infection*

A strain of *Bact. coli* isolated from the abnormal milk of a cow was grown in broth for four hours at 37° C.: 0·25 ml., containing 500×10^6 organisms per ml., was highly pathogenic to mice when given intraperitoneally.

The results of a preliminary trial to test the value of a single dose of antibiotics against this inoculum are shown in Table VIII.

It is clear that streptomycin was a most effective agent against *Bact. coli* while aureomycin also gave moderately good protection.

Penicillin and nisin which had no protective value were not used in a further experiment which was designed to show the relationship of number of organisms to the protection afforded (Table IX). The results confirm the preliminary experiment in showing that streptomycin is especially potent against *Bact. coli* and that aureomycin gives good protection against the smaller doses.

TABLE VIII

PROTECTIVE VALUE OF A SINGLE DOSE OF ANTIBIOTICS AGAINST BACT. COLI.

Antibiotic	*Dose*	*No. Mice*	*No. Survivors*
Penicillin G.	1 mg.	20	0
Aureomycin		,,	14
Nisin		,,	0
Streptomycin		,,	20
Control	—	15	0

TABLE IX

PROTECTIVE VALUE OF A SINGLE DOSE (1 mg.) OF AUREOMYCIN AND STREPTOMYCIN AGAINST VARYING NUMBERS OF BACT. COLI.

Density of broth culture	*No. of viable organisms*	*No. Mice*	*No. Survivors.*		
			Aureomycin	*Streptomycin*	*Control*
Brown tube 1/2	60×10^6	20	16	20	0
,, 1/2	90×10^6	,,	17	20	0
,, 2	300×10^6	,,	10	20	0
,, 2	500×10^6	,,	4	18	0

Salmonella dublin *Infection*

The strain used (Salm. 482) was isolated from the consolidated lung of a calf and was pathogenic for mice when 0·25 ml. of a four-hour Hartley broth culture was injected intraperitoneally; the number of organisms in such a culture was about 200×10^6 per ml. A preliminary experiment to test the value of a single dose of each antibiotic was first carried out (Table X).

TABLE X

PROTECTIVE VALUE OF A SINGLE DOSE OF ANTIBIOTICS AGAINST SALM. 482.

Antibiotic	*Dose*	*No. Mice*	*Death rate during seven days*							*No. Survivors*
			1	*2*	*3*	*4*	*5*	*6*	*7*	
Penicillin G.	1 mg.	20	0	5	7	8				0
Aureomycin		,,	0	0	3	6	1	1	5	4
Streptomycin		,,	0	2	6	9	1	2		0
Control	—	20	7	13						0

It is clear that a single dose was not effective in conferring protection on mice, but it will be noted that while death in the control mice occurred within two days it was delayed until after this time in the treated mice.

A further experiment was therefore carried out to test the value of multiple dosage administered as follows (*a*) by giving a second dose 48 hours after the first, (*b*) by giving four daily doses but with half the amount of antibiotic. The results obtained are shown in Table XI.

TABLE XI

PROTECTIVE VALUE OF REPEATED DOSES OF ANTIBIOTICS AGAINST SALM. 482.

Antibiotic	*Dosage*	*No. Mice*	*Death rate during seven days*							*No. Survivors*
			1	*2*	*3*	*4*	*5*	*6*	*7*	
Penicillin G.	2 × 1 mg.	20	0	2	4	4	9	0	1	0
Aureomycin	(at once and at	,,	0	0	0	0	6	7	0	7
Streptomycin	48 hours)	,,	0	1	4	3	4	5	2	1
Penicillin G.	4 × 0·5 mg.	20	7	5	7	0	1			0
Aureomycin	(daily)	,,	0	0	0	2	5	3	2	8
Streptomycin		,,	1	3	5	2	2	6	1	0
Control	—	20	4	16						0

It is significant in the above experiment that in addition to a better survival rate, deaths were delayed for the most part until after the first four days in mice treated with aureomycin whereas penicillin and streptomycin failed to confer this protection.

In view of the inefficiency of penicillin and streptomycin to overcome salmonella infection it was decided to compare the value of chloromycetin with that of aureomycin against varying numbers of organisms in the test dose. Chloromycetin is an antibiotic possessing activity against Gram-negative organisms and according to the findings of Smith, Joslyn, Gruhzit, McClean, Penner and Ehrlich (1948), it is more active *in vitro* than streptomycin against salmonella. It was found by Borolossy and Buttle (1949) to prolong life in mice infected with *Sal. typhi* when several doses were used. *In vitro* tests carried out in the present study showed that it possessed about the same activity against *Bact. coli* and *Sal. dublin* as aureomycin, both organisms being inhibited by a concentration of 5μg. per ml. in a broth medium.

TABLE XII

PROTECTIVE VALUE OF AUREOMYCIN AND CHLOROMYCETIN AGAINST SALM. 482 USING FOUR DAILY DOSES OF 0·5 mg.

Density of broth culture	*No. of viable organisms*	*No. Mice*	*No. Survivors*		
			Aureomycin	*Chloromycetin*	*Control*
Brown tube 1/6	30×10^6	20	9	0	0
„ 1/4	90×10^6	„	7	0	0
„ 1/2	120×10^6	„	2	0	0
„ 1	350×10^6	„	0	0	0

It can be seen in Table XII that while there was no protection with aureomycin against the highest test dose its value increased with diminishing size of dose. Chloromycetin on the other hand showed no protective power against even the lowest dose. It was noted that many mice treated with aureomycin and surviving seven days remained infected so that for efficient therapeutic purposes a dosage covering a longer period than four days would have to be instituted against such an infection.

RELATIONSHIPS BETWEEN THE *IN VITRO* SENSITIVITY OF ORGANISMS AND THEIR SUSCEPTIBILITY *IN VIVO*

It has been shown that it may be necessary to administer different dosage of the antibiotics in order to secure a reasonable survival rate against different experimental infections. For instance, against a virulent Group C streptococcus it was necessary to use four doses at short intervals, against salmonella infection four doses at daily intervals and against coliform and staphylococcal infection a single dose proved adequate to confer partial to complete protection.

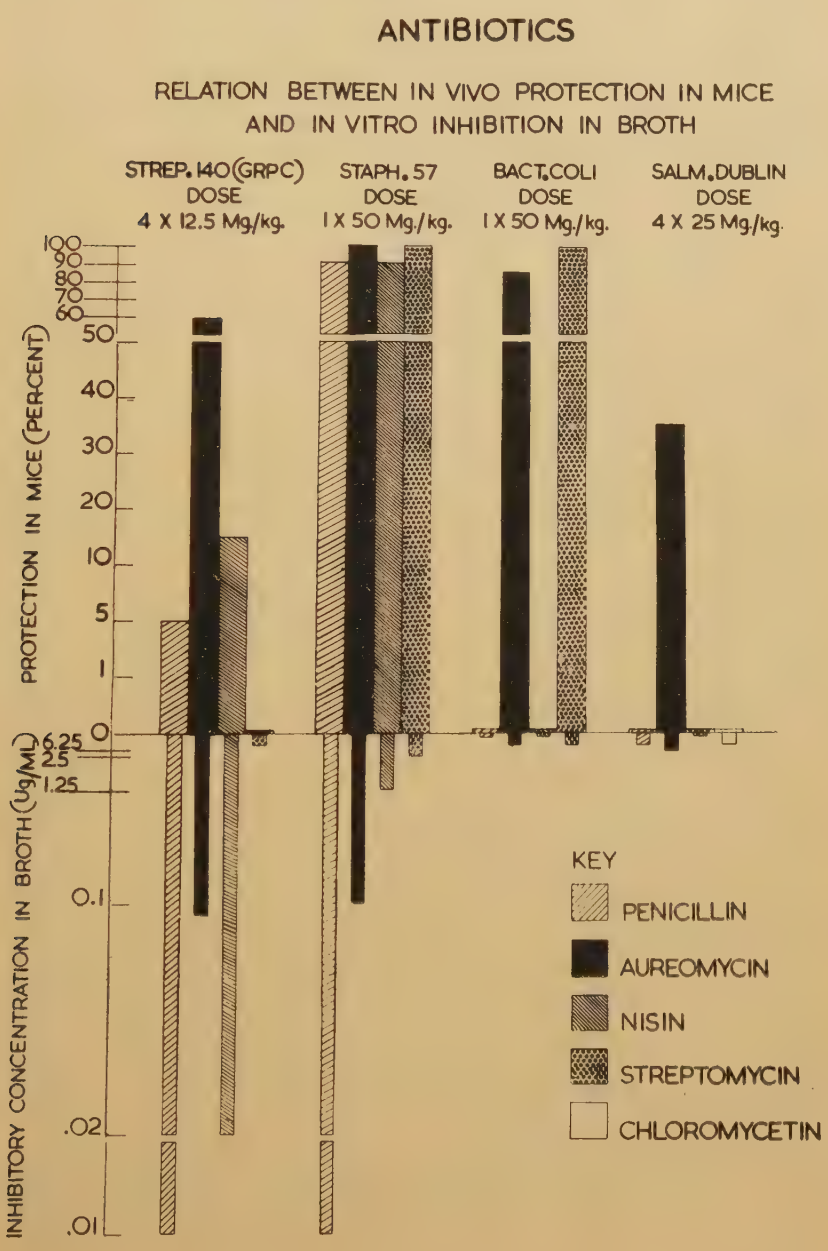
The degree of protection further depends on the number of organisms in the test inoculum and the value of individual antibiotics may vary from little or none to complete protection in inverse relation to the number of organisms injected. These variations have served to emphasise the fact that any attempt at assessing the relative value of the antibiotics *in vivo* and comparing such potency with their *in vitro* properties must depend in the first place on the selection of an infective dose of known bacterial count. For this reason a dose of moderate count and virulence containing 100×10^6 organisms per ml. has been chosen.

The results of such a comparison are depicted in Fig 1, which is intended to show the percentage survival rate conferred in mice by standard doses of the antibiotics in relation to the sensitivity

of the organism in a broth medium expressed as a reciprocal of the inhibitory end-point.

It will be seen that there is no strict correlation between *in vitro* activity and *in vivo* protection in mice. Penicillin is the most active antibiotic *in vitro* against the Group C streptococcus

FIG. 1



being ten times as potent as aureomycin and twice as potent as nisin and yet it is surpassed by the two latter in combating infection in mice. While there is a wide variation in the sensitivity of the staphylococcus strain *in vitro* from 0·01 μg./ml. to penicillin to 2·5 μg./ml. to streptomycin, the *in vivo* potency of the four antibiotics is almost identical. The strain of *Bact. coli* showed comparative resistance *in vitro,* being inhibited only by high concentrations of aureomycin and streptomycin and yet this strain was especially susceptible in mice to these two antibiotics. The salmonella strain showed a moderate sensitivity to penicillin, chloromycetin, and aureomycin but only the last of these antibiotics proved of any value against infection in mice.

DISCUSSION

Two methods are commonly employed for expressing the potency of antibiotics against pathogenic organisms, the first being the inhibitory action on the organism in various media, the second the protective action conferred against experimental infections. Throughout the literature, however, there has been a tendency to place reliance on the *in vitro* method in the selection of the antibiotics for therapeutic purposes while the second method has not been extensively used for confirmation.

There are many disadvantages to laboratory methods of assaying bacterial sensitivity and these have been recently summarised by Bliss (1951). It is generally agreed that a standard method should be used so that all workers may refer to the sensitivity of the different organisms under the same conditions. Of the methods now employed, a serial dilution method in a liquid medium is to be preferred because an exact end-point can be obtained whereas in a solid medium, using the cylinder or ditch method, no such precision is possible and there is the added disadvantage that antibiotics may not diffuse equally into the surrounding medium. Again, in a liquid medium, the criteria for judging the comparative potency of antibiotics should be based on the bacteriostatic rather than on the bactericidal sensitivity of organisms. This aspect has been clearly demonstrated in the present investigation where it has been shown that aureomycin compares very unfavourably with penicillin when judged by its lethal effect on organisms *in vitro*.

The technique employed in this present study for investigating the *in vivo* efficiency of the antibiotics was similar to that adopted by Bryer *et al.* (1948) and by Long *et al.* (1950). The dosage of antibiotic was given in sufficient amount to reveal differences in efficiency and not to develop the maximum therapeutic effect of any antibiotic.

It is clear from the present results that the *in vivo* method calls for a standard technique in assessing the value of antibiotics. Just as the end-point given by the *in vitro* technique depends on the

number of organisms in the medium, so the protective action of the antibiotics *in vivo* bears a delicate relationship to the number of organisms in the test inoculum. This being the case, it is clearly difficult to correlate the results of *in vitro* and *in vivo* tests since an organism known to be sensitive *in vitro* may be destroyed when small numbers are inoculated into mice protected by this antibiotic but may cause death when injected in large numbers for this reason it is suggested that when a comparison is made of the *in vivo* potency of several antibiotics with their *in vitro* action a standard test dose of culture containing about 100×10^6 organisms should be employed in the test inoculum. When this is done the two methods of assessing the antibiotics do not agree sufficiently to be able to predict their protective action in mice from the *in vitro* sensitivity of the organism under test.

It is suggested, therefore, that the selection and use of antibiotics for therapeutic purposes against a particular infection should rationally be based on the following data: —

(a) the determination of which antibiotics possess an inhibitory action on the causal organism *in vitro*.

(b) a further selection of these antibiotics as shown by their efficiency against experimental infection in a susceptible animal when subjected to a dose of known bacterial count.

(*c*) the method of application of the selected antibiotic, i.e. the route and interval of injection, should be based on a knowledge of its retention in the animal body.

SUMMARY

A study of the *in vitro* and *in vivo* activity of penicillin, aureomycin, nisin and streptomycin against various Gram-positive and Gram-negative organisms associated with bovine mastitis was made. Their action, as well as that of chloromycetin, was also tested against a strain of *Salm. dublin*.

Pencillin alone of the antibiotics tested possessed marked bactericidal activity especially against Gram-positive organisms.

When the bacteriostatic action of these antibiotics was assessed by a serial dilution method in Hartley broth, the Gram-positive organisms were sensitive in various degrees to penicillin, aureomycin and nisin but were comparatively resistant to streptomycin. Of the Gram-negative organisms, *Bact. coli* was sensitive only to aureomycin and streptomycin while *Salm. dublin* was inhibited by aureomycin and chloromycetin.

The *in vivo* action of the antibiotics was tested against four different experimental infections in mice using a standard dose of 50 mg./kg. or a variation of this dose at the time of injecting culture.

The protective action of the antibiotics against these infections was inversely related to the number of organisms in the test inoculum.

There was no strict correlation between the *in vitro* sensitivity of organisms and susceptibility *in vivo* when a standard dose of 100×10^6 organisms per ml. was inoculated into mice. For this reason it is suggested that an *in vivo* test should be used to confirm the selection of antibiotics for therapeutic use in clinical infections.

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STUDIES ON VITAMINS A AND C IN BOVINES

THE SEASONAL VARIATION IN THE VITAMIN A RESERVES OF COWS

By

James Stewart and J. W. McCallum

Animal Diseases Research Association, Moredun Institute, Gilmerton, Edinburgh

AND

P. S. Watts

Hannah Dairy Research Institute, Kirkhill, Ayr.

INTRODUCTION

In previous work (Stewart and McCallum, 1942) it was shown that despite feeding large vitamin A supplements in the form of carrots or cod-liver oil to dairy cows during pregnancy, the vitamin A content of the colostrum was not significantly altered. It had been presumed that on dry dairy rations of hay and concentrates the liver reserves of vitamin A would steadily decrease during a winter pregnancy and that the addition of potentially vitamin-rich supplements would keep liver reserves at a high level and thus increase the vitamin A content of the colostrum. Though later work by Spellman, Thomas, Lossli, Whiting, Norton and Turk (1947) using much larger vitamin A or carotene supplements (1 million I.U. vitamin A or 1 million I.U. carotene daily for 60 days) suggested that the vitamin A or carotene content of the prepartum diet could influence the appearance of these constituents in the colostrum, yet there are suprisingly little data in the literature concerning the seasonal variation in liver reserves of vitamin A during winter or summer feeding or of the effects of a winter or summer pregnancy on the liver reserves of vitamin A at parturition. This is perhaps understandable in the light of the high prices of dairy cows, which would make research workers hesitate before sacrificing even experimental animals to obtain this information.

Braun (1945) found that the vitamin A content of the liver varied according to diet, but that if a shark-liver oil supplement was fed it reached a common level, regardless of the basic diet. He deduced from this that there was an optimum level for vitamin A storage. Wise, Atkeson, Caldwell, Parrish and Hughes (1947), also as a result of prolonged vitamin A feeding experiments, concluded that differences in vitamin A intake were reflected in the concentration of vitamin A in the liver though not necessarily in the concentration of vitamin A in the blood.

METHODS

In a previous paper, Watts (1950) discussed the vitamin C levels in the liver, kidney and plasma of a population of cows passing through a public abattoir. The opportunity was taken of estimating the vitamin A and carotene contents of the livers of these cows. Immediately after slaughter a sample of the liver was placed in a tin, which was hermetically sealed and dispatched by train to Moredun Institute,, where it arrived within six hours of the death of the animal.

The vitamin A and carotene contents of the liver were immediately assayed by the method of Davies (1933), the final readings being read on a photocell absorptiometer. In the original design of the experiment it was hoped that livers from six cows would be received weekly, but it was not always possible to keep to this number and occasionally more than six were received. The investigation started in September, 1946, and finished in June, 1949, with intermittent gaps when, for various reasons, livers could not be obtained or assays be carried out. In all 657 livers were examined.

As related by Watts (1950), attempts were made to trace all the animals slaughtered and to obtain information regarding feeding and management, as well as details of age, pregnancy and state of health. In many cases it was not possible to procure this information and in others the information was not complete or reliable.

RESULTS

The results obtained were calculated on a monthly basis and are recorded in Table I.

The most striking feature was the large seasonal variation. The lowest values each year occurred during the months of April and May and the highest during November and December. The results for total vitamin A are shown graphically in Fig. 1, where it is clearly demonstrated that during the four years under investigation there was a sharp fall during February and March. The April values for 1946, 1947, 1948 and 1949 were 91, 86, 88, 114 I.U./g. respectively as compared with values for November of 1945 and 1948 of 324 and 484 I.U.g. respectively.

As seen in Table I this seasonal fall affected the carotene as well as the vitamin A. Braun (1945) found the first demand on liver reserves affected carotene only, which is converted to vitamin A, thus depleting the carotene content of the liver, but without decreasing the vitamin A level. In the present investigation, however, both vitamin A and carotene contents of the liver showed very similar trends, with maximum values at the same season and a decline in both constituents during the same period.

TABLE I

SHOWING MEAN CONCENTRATION OF VITAMIN A IN LIVER SAMPLES.

Month and Year		*No. of Cows*	*Vitamin A I.U./g.*	*S.E.*	*Carotene as Vitamin A I.U./g.*	*S.E.*	*Total Vitamin A I.U./g.*	*S.E.*
September,	1945	28	99	±13·49	17·0	± 2·99	116	±15·68
October	,,	20	195	±14·52	30·0	± 3.54	225	±16·78
November	,,	34	275	±22·37	49·0	± 2·91	324	±24·01
December	,,	12	256	±23·83	38·0	± 2·78	294	±25·02
January,	1946	23	209	±21·27	24·0	± 2·68	233	±22·78
February	,,	23	146	±14·34	17·5	± 1·71	164	±15·63
March	,,	20	192	±24·93	14·4	± 2·09	206	±26·19
April	,,	12	86	±19·97	5·5	± 0·07	92	±20·29
May	,,	12	36	±11·74	6·5	± 1·85	42	±12·84
June	,,	30	144	±13·96	23·8	± 1·42	168	±14·67
July	,,	15	188	±12·56	25·6	± 3·44	144	±14·78
September	,,	17	156	±12·55	34·1	± 2·40	190	±14·02
October	,,	29	193	±12·31	43·3	± 1·25	236	±13·20
November	,,	12	167	±18·37	37·1	± 1·84	204	±19·98
February,	1947	11	64	± 5·40	6·1	± 0·04	70	± 5·48
March	,,	23	82	± 3·70	7·4	± 0·05	89	± 4·02
April	,,	20	78	± 4·98	8·6	± 0·09	86	± 5·29
May	,,	10	64	±10·50	11·0	± 2·32	75	±12·42
June	,,	18	74	± 6·43	18·2	± 1·78	92	± 7·98
July	,,	12	85	± 6·55	23·5	± 2·37	109	± 6·02
March	1948	15	206	±38·27	6·2	± 1·21	212	±39·13
April	,,	17	83	±21·22	5·9	± 1·41	89	±22·21
May	,,	20	170	±30·88	13·6	± 2·16	184	±32·80
June	,,	17	237	±23·30	18·0	± 1·34	255	±23·64
July	,,	10	238	±21·09	21·4	± 1·36	259	±24·05
September	,,	5	375	±86·67	15·6	± 3·55	391	±90·08
October	,,	26	318	±24·21	25·7	± 1·82	344	±25·09
November	,,	32	448	±33·48	36·2	± 2·25	484	±34·91
December	,,	22	395	±36·52	45·2	± 2·37	440	±38·22
January,	1949	18	340	±55·52	12·8	± 1·75	353	±57·00
February	,,	17	273	±36·56	10·8	± 1·39	284	±37·51
March	,,	22	137	±19·83	6·2	± 0·07	143	±20·37
April	,,	18	111	±18·40	3·6	± 0·07	115	±18·86
May	,,	22	171	±21·85	10·3	± 1·27	181	±21·97
June	,,	15	164	±19·57	14·5	± 1·54	179	±20·84
Total 35 months		657	191	± 5·80	20·8	± 0·06	213	± 6·12

The maximum reserves were noted within a month or six weeks of the transference of the cows from outside grazing to indoor conditions and at a time when they were fed winter rations low in vitamin A such as turnips, hay and concentrates. The minimum values occurred during April, just after the cows had been transferred from their stalls to pasture, and it was only after the animals had been six weeks at pasture that the vitamin A reserves in the liver began to rise. The results for 1947 do not agree so closely with the trend of the other years since when sampling started in February, 1947, the reserves were already low and this low concentration was maintained till June, when a rise was apparent. Weather conditions during late winter and

FIG. 1

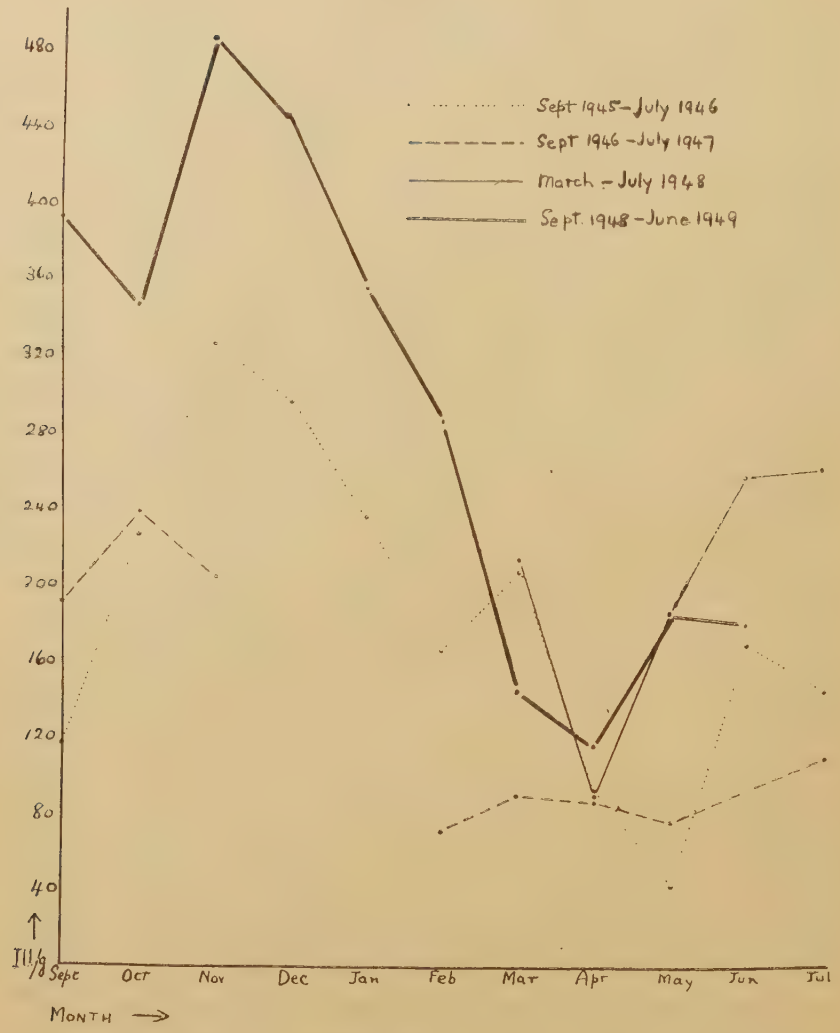

FIGURE 1. Monthly variations in liver vitamin A.

early spring in 1947 were of the severest type, temperatures remained low over prolonged periods and heavy snowstorms lasted from Christmas till well into April. Even housed animals were adversely affected by the weather, losing body weight although fed standard rations which normally allowed of progressive live weight increases.

It is considered possible that the large seasonal variations and the times of the maxima and minima reserves might be attributed to environmental conditions, especially that of temperature, since the removal to a warm house after the more chilly conditions of October in the field might account for the maxima

occurring in November. The low values for April and May might be due to the transference of the animals from warm stalls to the colder outside temperatures. Keener, Bechdel, Guerrant and Thorp (1942) showed a marked lowering of blood carotene and blood vitamin A in calves during cold weather and noted that the minimum requirements for vitamin A for calves in cold weather might be twice as great as during warm weather. Koehn (1943) showed that the *immediate* effect of grazing was to increase the vitamin A activity from 666·7 I.U. to 3,500 I.U. per quart of milk within a fortnight, thus probably utilizing liver reserves, but he does not state the weather conditions under which the grazing was started. Koehn's statement is in general agreement with the accepted opinion that the summer values for the vitamin A content of butterfat are substantially higher than the winter values. Average values given by the U.S. Department of Agriculture (1947) are 23·1 I.U./g. for winter and 37·5 I.U./g. for summer. It is unfortunate that such studies have not related the vitamin A content of the milk to the liver reserve values of vitamin A.

The results reported in the present series were obtained from a population of cows slaughtered at a public abattoir. Although they may not be considered average for cows on a farm it is of interest that during four successive years the trend was identical despite differences in composition of diet and management. It is very probable, therefore, that the results of Table I do indicate what is happening in dairy herds kept under similar conditions and that during the winter months the liver reserves of vitamin A are relatively high. Consequently during January and February cows were calving when liver reserves were high and this may be the reason why there was no appreciable effect on the vitamin A content of the colostrum after several months of feeding a vitamin A rich supplement (Stewart and McCallum, 1942). In order to ensure high contents of vitamin A in the colostrum it would appear that the vitamin A metabolism should be stimulated rather than the dietary vitamin A potential should be increased.

As mentioned at the beginning of the paper details regarding management, feeding and health of the animals were obtained where possible, but the large seasonal variation makes a strict interpretation of these factors in relation to the vitamin A liver reserves very difficult. In Table II the animals have been grouped under six classifications.

The difference between the mean values for "cows'" and "heifers", 220 I.U./g. as compared with 130 I.U./g., is significant but the number of heifers *viz.* 30, is far smaller than that in the cow class *viz.* 505. Dann (1933) stated that the vitamin A content of the colostrum of heifers was higher than that of other cows, but Stewart and McCallum (1938) were unable to confirm this. The difference in liver reserves of vitamin A between the classes

TABLE II

VITAMIN A IN COWS' LIVERS.

Class	*No.*	*Vit. A. I.U./g.*	*Carotene I.U./g.*	*Total Vit. A. I.U./g.*
Cows	505	203·0	17·9	220·9
Heifers	30	114·4	15·6	130·0
Pregnant	127	213·9	24·9	238·8
Non-pregnant ...	294	182·5	19·4	201·9
At pasture	269	195·3	19·9	215·2
Housed	149	182·2	15·3	197·5
Total	657	191·9	20·8	212·7

"pregnant" and "non-pregnant" was not significant, which was to be expected since the livers of calves at birth usually contain no vitamin A and therefore the vitamin A reserves of the mother are not being utilised by the foetus.

The other two classes, "housed" and "at pasture", shown in Table II, also exhibited no significant difference, but this was probably due to the large seasonal variation and the fact that the cows were seven months at pasture, *i.e.*, from April to October, and that by June the liver reserves were being restored to quite high levels.

SUMMARY

The livers of 657 cows, slaughtered at a public abattoir from September, 1945, to June, 1949, were assayed for carotene and vitamin A. They showed a marked seasonal variation in vitamin A reserves. The maximum values (300 to 400 I.U./g.) occurred in November-December, when the cows were on winter rations, and the minimum (75 to 90 I.U./g.) in April-May, within a short time of the animals being transferred from stalls to pasture.

The mean concentration of vitamin A in the 657 livers was 213±6·12 I.U./g.

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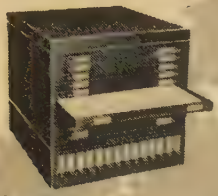

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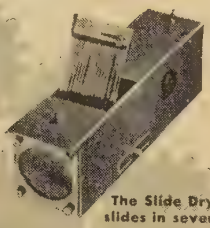

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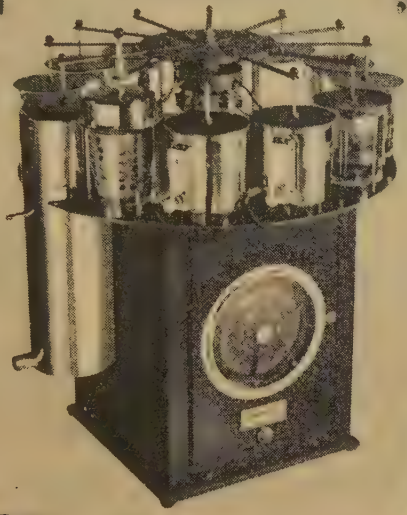

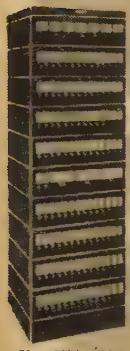

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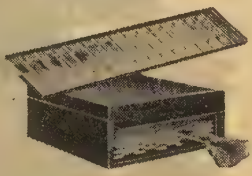

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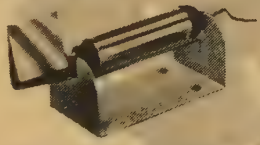

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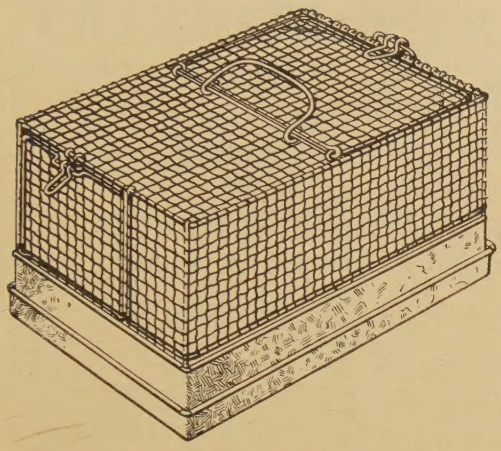

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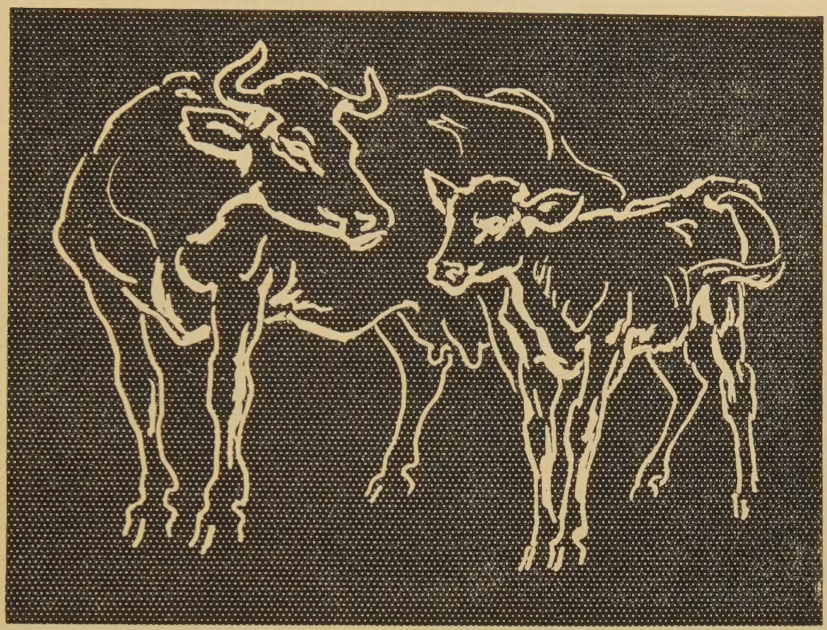

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